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# autoMACS<sup>®</sup> Pro Separator

## User manual

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**Thank you for choosing a Miltenyi Biotec product.**

The autoMACS® Pro Separator is an innovative instrument for automated sensor-controlled multisample labeling and separation of various cells types. At the touch of a button, target cells are magnetically labeled, separated, and eluted in a fully automated fashion.

Purify and progress.



**Please read before use!**

**Please read all information contained in this user manual before use. Failure to read and follow these guidelines could lead to improper or incorrect use, handling or care of your instrument and could cause hazards to users, unpredictable results, instrument malfunction or damage, premature wear and reduced life time of the instrument, and may void your warranty.**

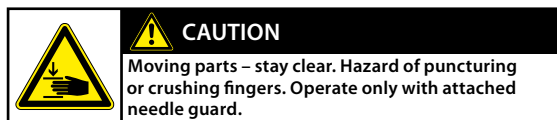
Keep this user manual in a safe place, accessible for anyone using the autoMACS Pro Separator.

This chapter describes the safety instructions and site requirements for your autoMACS Pro Separator. The following warnings and cautions are provided to help you prevent injury to yourself or damage to the instrument.

## 1.1 Symbols and hazard levels

### Setup of safety notices

#### Example



The safety notices inform the user about potential risks if warnings and precautions outlined below are not followed. The icon on the left side specifies the risk. The hazard level at the top classifies the hazard, as mentioned above. The level, type, and source of the hazard as well as potential consequences, prohibitions, and measures are pointed as follows.

### Symbols and hazard levels

The following chart is an illustrated glossary depicting the symbols that are used in this user manual and on the AutoMACS Pro Separator.



Indicates a hazard situation, which if not avoided, could result in minor or moderate injury.



Indicates a hazardous situation which, if not avoided, could result in death or serious injury.



Attention, consult the User Manual for further instructions and proceed with caution.

Warnings include the risk of damage to the equipment, severe personal injury, or loss of life



Hazard of crushing and shearing.  
Risk of crushing and shearing of bodily parts due to mechanical hazards.



Laser radiation  
Risk of serious eye and skin injuries.



Strong magnetic field  
The magnetic field can interfere with magnetizable objects and electronic devices or damage magnetic information carriers. Risk of severe personal injury to persons carrying pacemakers or electronic medical implants.



Risk of contamination if biohazardous material is used. Indicates the risk of loss of life, severe injury to the instrument operator, or equipment damage due to potentially dangerous biological material.



Indicates the risk of loss of life or severe injury to the instrument operator due to hazardous voltage.



Protective conductor terminal  
Symbol is attached on the inside of the instrument. Warning for service personnel.



On (supply)

Off (supply)



Documentation needs to be consulted before proceeding with installation and operation of the system.

## 1.2 Warnings and precautions

The autoMACS Pro Separator employs state-of-the-art technology. It is a computer-controlled instrument for the automated separation of magnetically labeled cells using MACS Technology. The MACS MiniSampler connects to the autoMACS Pro Separator and thus represents a part of the cell separation instrument. The autoMACS Pro Separator and the MACS MiniSampler are designed to operate safely after installation and when used by trained personnel according to general safety practices and the instructions set forth in this user manual. The guidelines in this section explain the potential risks associated with the operation of the instrument and provide important safety information in order to minimize these risks. By carefully following the instructions, you can protect yourself and the equipment from potential hazards and create a safe work environment. If this instrument is used in a manner not specified by the manufacturer, protection may be impaired.

**IMPORTANT:** Please read and follow all operating instructions in this user manual and pay attention to all warnings displayed on the instrument. Retain this user manual and any other safety and operating instructions provided with the instrument in a place accessible to all users for future reference.


**IMPORTANT:** The autoMACS Pro Separator is intended for indoor use only. Do not use the instrument in areas classified as hazardous locations such as oxygen-laden environments.

Contact your local authority governing electrical power supply, building constructions, maintenance, or safety for more information regarding the installation of the equipment.

## 1.3 General precautions

To reduce potential risks associated with operating the autoMACS Pro Separator, please observe the following general precautions. Failure to observe these precautions could result in fire, bodily harm, and/or damage to the instrument.

### 1.3.1 Hazard of electric shock and spread of fire

	<p><b>WARNING</b></p> <p>Hazardous voltages. Risk of loss of life severe personal injury. Unplug before cleaning. Do not use</p> <ul style="list-style-type: none"> <li>- if device is opened or damaged,</li> <li>- if liquids have been spilled into device,</li> <li>- if objects device through ventilation slots.</li> </ul>
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**WARNING:** Electrical devices pose the risk of an electric shock.

To reduce the risk of an electric shock, do not open any cover other than the front access covers of the autoMACS Pro Separator nor any other accessory hardware supplied by Miltenyi Biotec.



All other covers of the instrument and accessory hardware are to be removed by authorized personnel only. Special care must be taken while handling fluids. Clean up spillages immediately. Do not allow fluids to enter the interior of the instrument. Unplug the power cord before manually cleaning the autoMACS Pro Separator.

**WARNING:** A potential risk exists if an opened, dropped or damaged autoMACS Pro Separator is used, if liquids are spilled into the instrument, if an object has entered the instrument through the ventilation slots, or if an object has been dropped into the instrument. If flames or smoke appear immediately switch off the the autoMACS Pro Separator, unplug the instrument from the electrical outlet, and contact an authorized Miltenyi Biotec service provider or the Miltenyi Biotec Customer Support team. Use of a damaged instrument or an instrument with a damaged power cable is expressly prohibited.

### 1.3.2 Strong magnetic field



**WARNING:** The autoMACS Pro Separator is equipped with an extremely powerful magnet. Keep any magnetic information carriers (such as credit cards, magnetic tapes and floppy disks), any electronic equipment (such as hearing aids, pacemakers, measuring and control instruments, computers, and watches) and magnetizable tools and objects at a distance of at least 20 cm from the magnet cover. These items may be affected or damaged by the magnetic field.



Figure 1.1 Location of warning sign for strong permanent magnet.

### 1.3.3 Hazard of crushing and shearing



**CAUTION:** Do not open the front access covers while the instrument is in operation. Do not obstruct the movement of the automated arm and accessory hardware during operation. Keep fingers etc. away from all moving parts of the autoMACS Pro Separator and accessory hardware, to avoid crushing or shearing injuries, or damage to the instrument. Do not touch fluid pumps or adjust the tubing, while the instrument is in operation. Always switch off the instrument before adjusting any part of the fluidic system. Always stop or abort a procedure before handling accessory hardware, e.g. MACS MiniSampler, or loading/removing tubes from the tube rack placed on the sampler. Do not circumvent any safety measures or devices.



Figure 1.2 Open circle shows warning sign for 'hazard of crushing and shearing'.

### 1.3.4 Laser radiation

**WARNING:** The instrument is equipped with four vertical cavity surface emitting lasers (VCSELs) for automated rack detection (Class 1M). The radiation is not visible. Do not view directly with optical instruments (e.g. lenses, magnifying glasses, and microscopes). Viewing the VCSEL port within 100 mm distance with optical instruments could be hazardous to the eye.

The instrument is also equipped with a 2D Code Reader which uses a visible semiconductor laser as a target pointer for adjusting the reading position and powerful light emitting diodes (LEDs) for illuminating the reading area.

Do not look directly at laser or LED radiation or reflected laser or LED radiation from a mirrored surface. Otherwise, eye injury may result. Do not intentionally direct the laser beam at others.

Do not disassemble, modify or remove the installed laser or LED radiation sources or their mounting brackets. The laser or LED radiation sources do not automatically stop emitting when disassembled.

Radiation of disassembled units may lead to eye injuries.

Be careful of the path of the laser beam or reflection from a mirrored surface. Take care during installation of the autoMACS Pro Separator that the path of the laser beam is not at the same height as that of the human eye during operation.

Do not allow water, oil, dust, or other foreign substances to stick to 2D Code Reader aperture window. This may cause read errors. Be sure to stop the laser emission before cleaning the scanner. Otherwise, exposure to the laser may cause eye injury. Use a soft, dry cloth to wipe any substances from the scanner. Do not use alcohol or other cleaning substance.

The autoMACS Pro Separator is classified as a Class 1M laser product per standard IEC 60825-1:1993+A1:1997+A2:2001.

**CAUTION:** Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

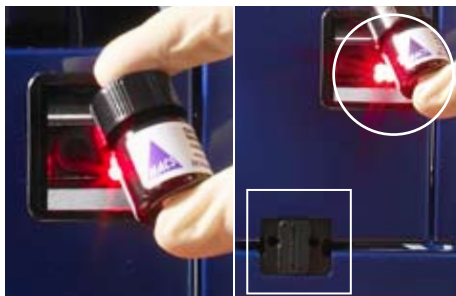


Figure 1.3 Position of lasers. Invisible rack detection lasers are located within the rectangle area. The 2D code reader (visible) is located within the open circle.



## 1.4 Secure installation

This section describes the requirements your site must meet for safe installation and operation of your autoMACS Pro Separator. Read the instructions in this section and ensure that your site is properly prepared before you connect the instrument to its power source.

When planning your site layout and equipment locations, keep in mind the precautions described in this section to help avoid instrument failures and reduce the possibility of environmentally caused shutdowns.

**IMPORTANT:** At all times, local working area safety instructions, laboratory policies, and standards regarding laboratory health and safety and prevention of accidents must be adhered to.

#### 1.4.1 Mounting accessories

Do not place the autoMACS Pro Separator on an unstable table, cart, stand, tripod, or bracket. As a consequence, the instrument might fall down. This may cause serious bodily harm and/or serious damage to the instrument. Use only on a table, cart, stand, tripod, or bracket recommended by Miltenyi Biotec or sold with the instrument. Do not place the autoMACS Pro Separator within a built-in apparatus or a confined space such as a shelf rack unless the apparatus has been specifically designed to accommodate the instrument, proper ventilation is provided, and the mounting instructions for the instrument have been followed.

#### 1.4.2 Air circulation

The instrument should not be placed next to radiators, heat registers, stoves, or other pieces of equipment (including amplifiers) that produce heat. Allow sufficient air circulation around the autoMACS Pro Separator—at least 15 cm on all sides—during operation to ensure adequate cooling of the instrument. Prevent direct exposure of the instrument to sunlight. Slots and openings of the instrument are provided for ventilation and should never be blocked or covered, as these ensure reliable operation of the autoMACS Pro Separator and protect the instrument from overheating. Never push a foreign object through an opening into the instrument.

#### 1.4.3 Water and moisture

Do not use the instrument in a wet or damp location. Avoid high humidity or condensation and protect the machine against water splashes.

#### 1.4.4 Grounded (earthed) product

The instrument is equipped with a three-wire electrical grounding-type plug that has a third pin for grounding. This plug only fits into a grounded power outlet. This is a safety feature. Do not try to insert the plug into a non-grounded power outlet. If you cannot insert the plug into the outlet, contact your local electrician to replace the outlet.

#### 1.4.5 Power sources

The instrument should only be operated from a power source indicated on the product's electrical ratings label. If you have questions about the type of power source to use, contact your authorized Miltenyi Biotec service provider or local power company.

Do not use extension cords or power strips. Do not overload an electrical outlet. The overall system load must not exceed 80% of the branch circuit rating.

#### **1.4.6 Accessibility**

Make sure that the main switch as well as the connector for the power cable are easily accessible and located as close to the operator of the instrument as possible. If it is necessary to disconnect the power supply, unplug the cable from the power outlet.

#### **1.4.7 Peripheral devices**

Only peripheral devices that comply to UL 60950 are allowed to be connected to the RS232 connector labeled "COM". The connector labeled "RS232/AUX" is not in use. In addition, only original autoMACS Pro Equipment should be attached to the connectors labeled "External CAN", "CAN1", and "CAN2". The voltage levels on these connectors shall not exceed hazardous voltage levels of 30 V rms. and 42.4 V peak or 60 Vdc. Only the autoMACS Pro Bottle Sensor Cable should be attached to the "Bottle Sensor" connector.

Only a 2D code reader recommended by Miltenyi Biotec should be connected to the "RS232/BCR" connector. External laser devices connected to the connector labeled "RS232/BCR" have to comply with the standard IEC 60825-1. Only use connector cables less than 3 m in length.

### **1.5 Secure operation, maintenance, transport and disposal**

Observe the following instructions to ensure secure operation, maintenance, transport, and disposal of your autoMACS Pro Separator.

**IMPORTANT:** At all times, local working area safety instructions, laboratory policies, and standards regarding laboratory health and safety and prevention of accidents must be adhered to.

#### **1.5.1 Safe operation**

If the instrument is not working properly and instructions or messages on the display screen advise to contact technical service, secure operation is no longer possible. Immediately switch off the autoMACS Pro Separator, unplug the instrument from the electrical outlet, and contact an authorized Miltenyi Biotec service provider or the Miltenyi Biotec Customer Support team.

#### **1.5.2 Servicing**

**IMPORTANT:** Unless otherwise specifically noted in this User Manual or other Miltenyi Biotec documentation, do not service the autoMACS Pro Separator yourself. Servicing and repair must be performed by qualified service personnel. Improper or incorrect servicing or repair

of your autoMACS Pro Separator can cause hazards to users, lead to unpredictable results, instrument malfunction or damage, premature wear and reduced life time of the instrument, and may void your warranty.

**Inquire with your local Miltenyi Biotec representative about Miltenyi Biotec's extensive instrument service and support arrangements, or refer to [www.miltenyibiotec.com/support](http://www.miltenyibiotec.com/support).**


**IMPORTANT:** When replacement or spare parts are required, make sure that the service provider uses only genuine Miltenyi Biotec parts or third-party parts specified and recommended by Miltenyi Biotec. Using unauthorized replacement or spare parts can cause malfunction of the instrument and impair cell separation results. Miltenyi Biotec does not honor any warranty or accept any responsibility for instrument failure or damages resulting from the use of inappropriate replacement or spare parts. After completing any service or repair work, have your authorized Miltenyi Biotec service provider perform all safety checks required by the repair procedure to ensure that the instrument is in proper operational condition.

Only use options and upgrades recommended by Miltenyi Biotec.

### 1.5.3 Cleaning

Unplug the autoMACS Pro Separator from the outlet before cleaning. Do not use liquid or aerosol cleaning agents; always use a damp cloth.

### 1.5.4 Hazardous material

	<p><b>WARNING</b></p> <p>Risk of loss of life if biohazardous material is used. Wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Operate device in a safety hood. Decontaminate device after spillage of biohazardous material.</p>
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If biohazardous material have been used, the operator shall choose and wear personal safety equipment in accordance with warnings and precautions for the used substances. Wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Also protect mouth and nose as aerosols might leak from the system (e.g. Washing Station). Defective or inadequate safety equipment might endanger the operator. The autoMACS Pro separator shall be operated in a safety hood if hazardous or unknown materials are processed. If hazardous material has been used or spilled, care must be taken to thoroughly decontaminate the system. For details, refer to section 7.3.4.

Always inspect the fluidics system (complete tubing set, bottles and their closures, valves, columns, diluter and needles) before switching on the instrument. If leakage has been detected, replace all damaged

parts before switching on the instrument. If damaged parts cannot be replaced, unplug and do not use the instrument. Failure of parts containing biohazardous material or liquids that have been in contact with such material could cause a hazard.

Columns, tubes, and any other consumables that were in contact with biohazardous samples shall be autoclaved prior to disposal. Liquid waste shall be autoclaved or decontaminated using a disinfectant that is appropriate for the specific pathogen, e.g. 10% bleach, isopropyl alcohol, or 70% ethanol.

Waste disposal must be in accordance with any local regulations.



Figure 1.4 Warning signs for biohazard located on lower facing panel of the autoMACS Pro Separator (left) and top of autoMACS Pro Separator fluid bottle (right).

### Flammable

70% ethanol is used in Sleep and Store programs. The solvent is flammable. Therefore, keep the instrument away from fire.

### 1.5.5 Transport

The autoMACS Pro Separator should be transported with care in packaging specified by Miltenyi Biotec. Internal damage can occur, if it is subjected to excessive vibration or if it is dropped. If the instrument needs to be shipped back to the manufacturer for service, decontaminate the instrument from any hazardous material prior to shipment. If you have questions regarding proper decontamination or shipment, please contact Technical Support for assistance. Refer to section 7.3.4 for further information on instrument decontamination.

### 1.5.6 Instrument disposal

Please contact Technical Support for assistance if you wish to dispose of your instrument.

### 1.5.7 Electromagnetic compatibility

Changes or modifications of the equipment unless expressly approved by Miltenyi Biotec may void your authority to operate the equipment pursuant to 47 CFR §15.

**Note:** This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.





## À lire avant utilisation.

**Veillez impérativement lire toutes les informations fournies dans ce mode d'emploi avant d'utiliser l'appareil. Le non-respect de ces consignes peut donner lieu à une utilisation, une manipulation et une maintenance inappropriée ou incorrecte de votre appareil, ce qui pourrait mettre en danger les utilisateurs, fournir des résultats non attendus, entraîner le dysfonctionnement ou la détérioration de l'appareil, son usure prématurée et réduire la durée de vie de l'appareil, ainsi qu'annuler votre garantie.**

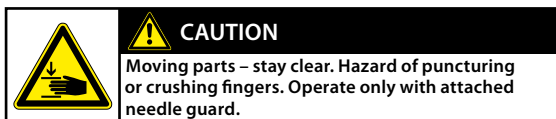
Conservez ce mode d'emploi dans un endroit sûr et accessible aux utilisateurs de l'unité de séparation autoMACS Pro® (séparateur autoMACS Pro).

Ce chapitre décrit les consignes de sécurité et les exigences d'installation s'appliquant à votre séparateur autoMACS Pro. Les avertissements et les mesures de précaution suivantes ont pour objectif de vous aider à éviter toute blessure corporelle ainsi que toute détérioration de l'appareil.

## 1.1 Symboles et niveaux de danger

### Mise en place des consignes de sécurité

#### Exemple



Les consignes de sécurité informent l'utilisateur des risques potentiels pouvant survenir si les avertissements et les mesures de précaution décrits ci-dessous ne sont pas respectés. Le pictogramme visible à gauche définit le risque. En haut, le danger mentionné précédemment est affecté à un niveau de danger. Le niveau, le type et la source du danger ainsi que les éventuelles conséquences, les interdictions et les mesures à prendre sont mentionnés conformément au tableau ci-dessous.

## Symboles et niveaux de danger

Le tableau suivant est un glossaire illustré expliquant les symboles utilisés dans ce mode d'emploi et sur le séparateur autoMACS Pro Separator.



Indique une situation dangereuse qui, si elle n'est pas évitée, peut entraîner des blessures mineures ou modérées.



Indique une situation dangereuse qui, si elle n'est pas évitée, peut entraîner la mort ou des blessures graves.



Attention, consultez le mode d'emploi pour obtenir plus de consignes et agissez avec prudence.

Les avertissements incluent le risque de détérioration de l'équipement, de blessure corporelle grave et le danger de mort.



Risque d'écrasement et de cisaillement.

Risque d'écrasement et de cisaillement des membres corporels dû à des dangers mécaniques.



Rayonnement laser

Danger de graves lésions oculaires et cutanées.



Champ magnétique puissant

Le champ magnétique interfère avec les objets magnétisables et les appareils électroniques ou endommage les supports d'information magnétiques. Risque de grave blessure corporelle pour les personnes portant un pacemaker ou des implants médicaux électroniques.



Risque de contamination en cas d'utilisation de matières nocives pour l'organisme. Indique le danger de mort, le risque de blessure grave pour l'opérateur de l'appareil ou le risque de détérioration de l'équipement dû à des matières biologiques potentiellement dangereuses.



Indique le danger de mort, le risque de blessure grave pour l'opérateur de l'appareil en raison de la tension dangereuse.



Borne pour conducteur de protection

Le symbole est apposé à l'intérieur de l'équipement. Avertissement destiné au personnel de maintenance.



Alimentation électrique activée

Alimentation électrique désactivée



La notice d'utilisation doit être consultée avant de procéder à l'installation et à l'utilisation du système

## 1.2 Avertissements et précautions

Le séparateur autoMACS Pro fonctionne avec une technologie de pointe. Il s'agit d'un appareil commandé par ordinateur conçu pour la séparation automatique des cellules à marquage magnétique à l'aide de la technologie MACS. Le mini-échantillonneur MACS est branché au séparateur autoMACS Pro et fait donc partie du dispositif de séparation des cellules. Le séparateur autoMACS Pro et le mini-échantillonneur MACS sont conçus pour fonctionner en toute sécurité après leur installation s'ils sont utilisés par des personnes qualifiées selon les pratiques de sécurité générales et les consignes mentionnées dans ce mode d'emploi. Les instructions de ce chapitre expliquent les risques potentiels liés à l'utilisation de l'appareil et fournissent des informations de sécurité importantes afin de réduire ces risques. Respectez strictement ces consignes pour protéger l'équipement et vous-même des risques potentiels et créer un environnement de travail sûr. La protection n'est pas garantie si cet appareil n'est pas utilisé conformément aux instructions données par le fabricant.

**IMPORTANT:** Veuillez lire et respecter toutes les instructions d'utilisation fournies dans ce mode d'emploi et observez tous les avertissements affichés sur l'appareil. Conservez ce mode d'emploi et les autres consignes de sécurité et d'utilisation fournies avec l'appareil à un endroit accessible pour tous les utilisateurs en vue de les consulter ultérieurement.

**IMPORTANT:** Le séparateur autoMACS Pro Separator est destiné à une utilisation intérieure uniquement. N'utilisez pas l'appareil dans des zones classées dangereuses telles que des environnements à teneur élevée en oxygène.

Pour de plus amples d'informations concernant l'installation de l'équipement, veuillez vous adresser à votre organisme local responsable de l'approvisionnement en électricité, des travaux de bâtiment, de la maintenance et de la sécurité.

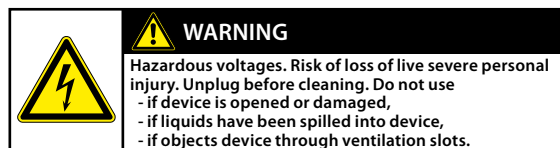
Si vous avez un doute sérieux quant à la sécurité d'utilisation de votre appareil, veuillez contacter votre prestataire de services Miltenyi Biotec agréé ou appelez le service après-vente de Miltenyi Biotec.

## 1.3 Précautions d'ordre général

Afin de réduire les risques potentiels liés à l'utilisation du séparateur autoMACS Pro Separator, veuillez observer les précautions générales

suivantes. La non-observation de ces précautions peut provoquer un incendie, des dommages corporels, et/ou détériorer l'appareil.

### 1.3.1 Risque d'électrocution et de propagation de feu



**AVERTISSEMENT:** Les appareils électriques présentent un risque d'électrocution. Afin de réduire le risque d'électrocution, n'ouvrez aucun cache du séparateur autoMACS Pro Separator, ni d'autres équipements accessoires fournis par Miltenyi Biotec. Tous les caches de l'appareil et des équipements accessoires doivent être démontés par le personnel agréé uniquement. Soyez particulièrement prudent pendant la manipulation de fluides. Nettoyez immédiatement si des liquides se sont déversés. Veillez à ce que les fluides ne s'infiltrant pas à l'intérieur de l'appareil. Débranchez le câble électrique avant de nettoyer manuellement le séparateur autoMACS Pro Separator.

Un risque potentiel existe si le séparateur autoMACS Pro Separator utilisé est ouvert, déformé ou endommagé, si des liquides se déversent dans l'appareil, si un objet est entré dans l'appareil par les fentes de ventilation ou si un objet a chuté dans l'appareil. En cas d'apparition de flammes ou de fumée, déconnectez immédiatement le séparateur autoMACS Pro Separator, débranchez l'appareil et contactez un prestataire de services Miltenyi Biotec agréé ou l'équipe de support technique de Miltenyi Biotec. Il est formellement interdit d'utiliser un appareil endommagé ou un appareil dont le câble électrique est endommagé.

### 1.3.2 Champ magnétique puissant



**AVERTISSEMENT:** Le séparateur autoMACS Pro est équipé d'un aimant extrêmement puissant. Veillez à laisser un espace d'au moins 20 cm entre le cache de l'aimant et les supports 'information magnétiques (cartes de crédit, bandes magnétiques et supports de données) ainsi que l'équipement électronique (appareils auditifs, pacemakers, dispositifs de mesure et de contrôle, ordinateurs et montres). Ces éléments peuvent être affectés ou endommagés par le champ magnétique. distance d'au moins 30 cm par rapport au matériel magnétique mentionné ci-dessus. Plus ils sont proches l'un de l'autre, plus la force attractive entre les deux aimants ou entre un aimant et

une matière magnétisable augmente. Par conséquent, n'approchez aucune matière magnétique – y compris tout autre aimant ou écran.



Figure 1.1 Symbole d'avertissement pour aimant puissant permanent.

### 1.3.3 Risque d'écrasement et de cisaillement



**Avertissement:** N'ouvrez pas les capots frontaux pendant le fonctionnement de l'appareil. Ne bloquez pas le mouvement du bras automatique ni des pièces correspondantes pendant le fonctionnement. N'approchez pas vos doigts, etc. des pièces mobiles du séparateur autoMACS Pro et des pièces correspondantes afin d'éviter toute blessure ainsi que toute détérioration de l'appareil due à l'écrasement et au cisaillement. Ne touchez pas les pompes à liquide et ne modifiez pas les tuyaux pendant le fonctionnement de l'appareil. Déconnectez toujours l'appareil avant de modifier une partie du système fluide. Stoppez ou annulez toujours le processus avant de manipuler les appareils supplémentaires comme le mini-échantillonneur MACS ou de charger/retirer les éprouvettes du râtelier à éprouvettes placé sur l'échantillonneur. Ne bloquez pas les dispositifs de sécurité et respectez les mesures de sécurité.



Figure 1.2 Le symbole d'avertissement entouré d'un cercle blanc indique un « risque d'écrasement et de cisaillement ».

### 1.3.4 Rayonnement laser

**AVERTISSEMENT:** L'appareil est équipé pour la détection automatique de porte-éprouvettes (classe 1M) par quatre diodes laser à émission par la surface à cavité verticale (VCSEL). Le rayonnement est invisible. Ne pas regarder directement dans ce rayon au travers d'instruments optiques (p. ex. lentilles, verres grossissants et microscopes). Regarder dans l'ouverture du VCSEL à une distance inférieure à 100 mm au travers d'instruments optiques peut endommager les yeux. L'appareil est également équipé d'un lecteur de code 2D utilisant un laser à semi-conducteur visible comme pointeur pour le réglage de la position de lecture ainsi que de diodes électroluminescentes (DEL) haute puissance pour l'éclairage du champ de lecture. Ne diriger le regard ni dans le rayon laser ou DEL direct, ni le rayon laser ou DEL réfléchi sur une surface de miroir. Sinon, des lésions oculaires peuvent en résulter. Ne pas diriger intentionnellement le rayon laser sur d'autres personnes. Ne pas démonter, échanger ni retirer des sources de rayon laser ou DEL encastrées ni leur support. Les sources de rayon laser ou DEL ne cessent pas forcément d'émettre un rayonnement une fois démontées. Le rayonnement d'appareils démontés peut entraîner des lésions oculaires. Prendre garde au chemin optique du rayon laser ou de sa réflexion sur une surface de miroir. Veiller lors de l'installation de l'autoMACS Pro Separator à ce que le chemin optique rayon laser ne se trouve pas à hauteur des yeux de personnes pendant le service. Eviter que de l'eau, de l'huile, de la poussière ou d'autres corps étranger adhèrent sur la fenêtre de lecture du lecteur de code 2D, comme ceci risque d'entraîner des erreurs de lecture. S'assurer avant le nettoyage du scanner qu'aucun rayon laser n'est plus émis. La manipulation du laser risque sinon de provoquer des lésions oculaires. Utiliser un chiffon doux pour essuyer des substances sur le scanner. Ne pas utiliser d'alcool ou d'autres produits de nettoyage.

L'autoMACS Pro Separator est désigné comme produit laser de la classe 1M selon la norme CEI 60825-1: 1993 + A1: 1997 + A2: 2001.

**AVERTISSEMENT:** l'utilisation d'organes de commande autres que ceux mentionnés dans ce document ainsi que toute adaptation ou utilisation d'autres procédés que ceux mentionnés dans ce document peuvent dégager des rayonnements dangereux.

Rayonnement laser

Danger de graves lésions oculaires et cutanées.



Figure 1.3 Position des lasers. Les lasers invisibles de détection de râteliers sont situés dans la zone rectangulaire. Le lecteur de code 2D (visible) est entouré d'un cercle blanc.



## 1.4 Sécurité de l'installation

Ce paragraphe décrit les exigences auxquelles votre site doit répondre pour garantir l'installation et la sécurité d'utilisation de votre séparateur autoMACS Pro Separator. Lisez les consignes de ce paragraphe et assurez-vous que votre site a été aménagé en conséquence avant de brancher l'appareil à sa source électrique. Lors de la planification de l'aménagement de votre site et de l'emplacement de l'équipement, tenez compte des précautions décrites dans ce paragraphe afin d'éviter que l'appareil ne tombe en panne et de réduire le nombre d'arrêts pouvant être causés par l'environnement de l'appareil.

**IMPORTANT:** Les consignes de sécurité concernant la zone de travail locale, les bonnes pratiques de laboratoire, ainsi que les directives relatives à la santé, à la sécurité et à la prévention des accidents en laboratoire doivent être observées en permanence.

### 1.4.1 Accessoires de montage

Ne placez pas le séparateur autoMACS Pro Separator sur une table, un chariot, un pied, un trépied ou un support. L'appareil pourrait sinon chuter, provoquant des blessures corporelles graves et/ou la détérioration considérable l'appareil. Utilisez le séparateur uniquement sur une table, un chariot, un pied, un trépied ou un support recommandé par Miltenyi Biotec ou vendu avec l'appareil. Ne placez pas le séparateur autoMACS Pro Separator dans un appareil intégré ou un espace restreint comme un casier, sauf si le dispositif a été



spécialement conçu pour accueillir l'appareil, qu'une ventilation appropriée est garantie et que les instructions de montage relatives à l'appareil ont été respectées.

#### **1.4.2 Circulation de l'air**

Ne placez pas l'appareil à proximité de radiateurs, de registres de chaleur, de fours ou d'autres pièces d'équipement (amplificateurs) qui produisent de la chaleur. Veillez à ce que suffisamment d'air puisse circuler autour du séparateur autoMACS Pro Separator — au moins 15 cm de chaque côté — pendant le fonctionnement afin de garantir le refroidissement adéquat de l'appareil. Évitez d'exposer l'appareil à un ensoleillement direct. Les encoches et les fentes de l'appareil sont prévues pour la ventilation et ne doivent jamais être bloquées ou recouvertes, car elles assurent le fonctionnement fiable du séparateur autoMACS Pro Separator et protègent l'appareil de la surchauffe. N'introduisez jamais d'objet étranger dans l'appareil par l'une des fentes.

#### **1.4.3 Eau et humidité**

N'utilisez pas l'appareil dans un endroit humide ou mouillé. Évitez l'humidité et la condensation et protégez la machine des éclaboussures.

#### **1.4.4 Produit relié à la terre**

L'appareil est équipé d'une fiche électrique trifilaire avec mise à la terre dont la troisième borne est prévue pour relier l'appareil à la terre. Cette fiche fonctionne uniquement dans une prise de courant avec contact de mise à la terre. Il s'agit d'un dispositif de sécurité. N'essayez pas d'insérer la fiche dans une prise de courant sans contact de mise à la terre. Si vous ne pouvez pas insérer la fiche dans la prise de courant, veuillez vous adresser à votre électricien local qui remplacera la prise de courant.

#### **1.4.5 Sources électriques**

Ne faites fonctionner l'appareil qu'à partir d'une source électrique indiquée sur la plaque signalétique du produit. Si vous avez des questions sur le type de courant électrique que vous pouvez utiliser, contactez votre prestataire de services Miltenyi Biotec agréé ou votre fournisseur d'électricité local. N'utilisez pas de rallonges ni de barrettes de connexion. Ne surchargez pas la prise électrique. La charge totale du système ne doit pas dépasser 80 % de la valeur nominale du circuit de dérivation.

#### **1.4.6 Accessibilité**

Assurez-vous que l'interrupteur principal et le connecteur du câble électrique soient facilement accessibles et placés aussi près que possible de l'opérateur de l'appareil. S'il s'avère nécessaire de déconnecter l'alimentation électrique, débranchez le câble.

#### **1.4.7 Appareils périphériques**

Seuls les appareils périphériques conformes à la norme UL 60950

peuvent être branchés au connecteur RS232 étiqueté « COM ». Le connecteur étiqueté « RS232/AUX » n'est pas utilisé. De plus, seuls des appareils autoMACS Pro originaux doivent être raccordés aux connecteurs étiquetés « External CAN », « CAN1 » et « CAN2 ». Le niveau de tension mesuré sur ces connecteurs ne doit pas dépasser le niveau de tension dangereux de 30 Veff ainsi qu'un pic de 42,4 V ou 60 V DC. Seul le câble de capteur de flacon autoMACS Pro peut être raccordé au connecteur étiqueté « Bottle Sensor ». Seul un lecteur de code 2D recommandé par Miltenyi Biotec peut être branché au connecteur « RS232/BCR ». Les appareils laser externes raccordés au connecteur étiqueté « RS232/BCR » doivent répondre à la norme IEC 60825-1. Utilisez uniquement des câbles de connexion de 3 mètres de longueur maximum.

## 1.5 Sécurité d'utilisation, maintenance, transport et élimination

Respectez les consignes suivantes afin de garantir la sécurité d'utilisation, la maintenance, le transport et l'élimination de votre séparateur autoMACS Pro Separator.

**IMPORTANT:** Les consignes de sécurité concernant la zone de travail locale, les bonnes pratiques de laboratoire, ainsi que les directives relatives à la santé, à la sécurité et à la prévention des accidents en laboratoire doivent être observées en permanence.

### 1.5.1 Sécurité d'utilisation

Si l'appareil ne fonctionne pas correctement et que les instructions ou les messages sur l'écran d'affichage conseillent de contacter le service technique, la sécurité d'utilisation de l'appareil n'est plus garantie. Déconnectez immédiatement le séparateur autoMACS Pro Separator, débranchez l'appareil et contactez un prestataire de services Miltenyi Biotec agréé ou l'équipe du service après-vente de Miltenyi Biotec.

### 1.5.2 Entretien et réparation

**IMPORTANT:** N'effectuez pas vous-même l'entretien du séparateur autoMACS Pro Separator, sauf autre consigne spécifique donnée dans ce mode d'emploi ou dans un autre document de Miltenyi Biotec. L'entretien et la réparation doivent être effectués par des réparateurs qualifiés. Tout entretien ou toute réparation inappropriée ou incorrecte de votre séparateur autoMACS Pro Separator peut mettre en danger les utilisateurs, fournir des résultats imprévisibles, entraîner le dysfonctionnement ou la détérioration de l'appareil, son usure prématurée et réduire la durée de vie de l'appareil, ainsi qu'annuler

votre garantie.

**Renseignez-vous auprès de votre représentant Miltenyi Biotec local sur les conditions d'entretien et de support complémentaires proposées par Miltenyi Biotec ou consultez le site [www.miltenyibiotec.com/support](http://www.miltenyibiotec.com/support).**


**IMPORTANT:** Si des pièces de remplacement ou de rechange sont requises, assurez-vous que le prestataire de services utilise exclusivement des pièces Miltenyi Biotec d'origine ou des pièces de fabricants tiers spécifiées et recommandées par Miltenyi Biotec. L'utilisation de pièces de remplacement ou de rechange non autorisées peut entraîner le dysfonctionnement de l'appareil et fausser les résultats de la séparation des cellules. Miltenyi Biotec n'accorde pas de prestation de garantie ou décline toute responsabilité pour les pannes et les dommages de l'appareil résultant de l'utilisation de pièces de remplacement ou de rechange inappropriées. Une fois les travaux d'entretien ou de réparation achevés, demandez à votre prestataire de services Miltenyi Biotec agréé d'effectuer tous les contrôles de sécurité requis par la procédure de réparation afin de garantir que l'appareil est parfaitement opérationnel.

Utilisez uniquement les options et les mises à jour recommandées par Miltenyi Biotec.

### 1.5.3 Nettoyage

Débranchez le séparateur autoMACS Pro Separator avant le nettoyage. N'utilisez pas d'agents nettoyants liquides ou en aérosol ; utilisez toujours un chiffon humide.

### 1.5.4 Matières dangereuses

	<p><b>WARNING</b></p> <p>Risk of loss of life if biohazardous material is used. Wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Operate device in a safety hood. Decontaminate device after spilling of biohazardous material.</p>
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Si une matière nocive pour l'organisme est ou a été utilisée, l'opérateur doit choisir et porter un équipement de protection individuelle conforme aux avertissements et aux précautions pour les substances utilisées. Portez des gants de protection, des vêtements de protection et des lunettes de sécurité afin d'éviter tout contact avec la peau et les yeux. Un équipement de sécurité défectueux ou inadéquat peut mettre l'opérateur en danger. Le séparateur autoMACS Pro Separator doit être manipulé dans un couvercle de protection si des matières dangereuses ou inconnues sont traitées. Si une matière dangereuse a été utilisée ou s'est déversée, prenez les précautions appropriées pour décontaminer soigneusement le système.

Les colonnes, les plaques, les tubes et tous les autres consommables qui ont été en contact avec des échantillons nocifs pour l'organisme doivent être traités à l'autoclave avant l'élimination. Les déchets liquides doivent être traités à l'autoclave ou décontaminés à l'aide d'un désinfectant adapté à l'agent pathogène spécifique, par ex. 10 % eau de Javel, alcool isopropylique ou 70 % d'éthanol.



Figure 1.4 L'élimination des déchets doit être effectuée conformément aux réglementations locales.

### 1.5.5 Transport

Le séparateur autoMACS Pro Separator doit être transporté avec soin dans un emballage spécifié par Miltenyi Biotec. Un dommage interne peut survenir si l'appareil est soumis à des vibrations excessives ou s'il chute. Si l'appareil doit être réexpédié pour être remis en état, décontaminez l'appareil afin d'éliminer toute matière dangereuse avant le transport. Si vous avez des questions concernant la propre décontamination ou l'expédition, n'hésitez pas à contacter notre service technique assistance.

### 1.5.6 Élimination de l'appareil

Si vous souhaitez éliminer votre appareil, veuillez contacter notre service technique qui vous aidera.

### 1.5.7 Compatibilité électromagnétique

**Pour les États-Unis uniquement.** Toute modification et toute transformation de l'équipement, sauf si elle a été formellement autorisée par Miltenyi Biotec, peut annuler votre droit d'utilisation de l'équipement conformément à la norme 47 CFR, art. 15.



## Bitte lesen vor Inbetriebnahme!

**Bitte lesen Sie vor Inbetriebnahme sorgfältig alle in diesem Benutzerhandbuch enthaltenen Informationen. Werden die folgenden Anleitungen nicht gelesen und beachtet, so könnte dies zu unsachgemäßer oder unvorschriftsmäßiger Anwendung, Bedienung oder Wartung des Gerätes führen. Auf diese Weise könnten Anwender gefährdet, Ihr Gerät beschädigt oder dessen Betrieb beeinträchtigt werden. Auch könnte sich vorzeitiger Verschleiß einstellen und somit die Lebensdauer des Gerätes verkürzen. Hierdurch könnten Ihre Garantieansprüche verfallen.**

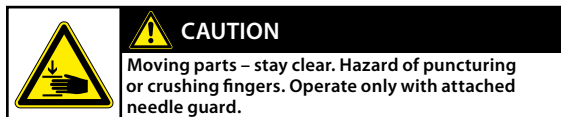
Bewahren Sie dieses Benutzerhandbuch an einem sicheren Ort auf, zugänglich für jeden Anwender der autoMACS® Pro Separator.

Dieses Kapitel beschreibt die Sicherheitsvorschriften und die Anforderungen an den Gerätestandort ihres autoMACS Pro Separator. Die folgenden Warnhinweise und Vorsichtsmaßregeln sollen Ihnen dabei behilflich sein, Verletzungen für sich oder Schäden am Gerät zu vermeiden.

## 1.1 Symbole und Gefahrenstufen

### Warnhinweis

#### Beispiel



Die Gefahrenhinweise informieren den Anwender über Gefahren, die bei Missachtung der nachstehend beschriebenen Warnungen und Vorsichtsmaßnahmen bestehen können. Das Gefahrensymbol links versinnbildlicht die Gefahr. Die Gefahrenwarnung oben bezeichnet die Schwere der vorliegenden Gefahr, wie vorstehend beschrieben. Schwere, Art und Ursprung der Gefahr sowie

mögliche Folgen, Verbote und Vorsichtsmaßnahmen werden im Anschluss beschrieben.

### Symbole und Gefahren Ebenen

Im folgenden tabellarischen Glossar werden alle in diesem Benutzerhandbuch oder auf dem autoMACS Pro Separator verwandten Symbole bildlich dargestellt und erklärt.



Bezeichnet eine Gefahrensituation, die, falls sie nicht vermieden wird, zu leichten oder mittelschweren Verletzungen führen kann.



Achtung, schlagen Sie im Benutzerhandbuch für weitere Anweisungen nach und gehen Sie umsichtig vor.



Warnhinweise deuten auf das Risiko von Beschädigungen des Gerätes bzw. der Ausrüstung, die Gefahr von schweren Verletzungen oder Lebensgefahr hin.



Quetsch- und Schergefahr.  
Durch mechanische Gefährdungen können Brüche oder Quetschungen von Körperteilen verursacht werden.



Laserstrahlung  
Gefahr von schweren Augen- und Hautverletzungen.



Starkes Magnetfeld  
Das Magnetfeld kann auf andere, magnetisch aufladbare Gegenstände und elektronische Geräte störend einwirken oder magnetische Datenträger beschädigen. Für die Träger eines Herzschrittmachers oder von elektronischen Implantaten besteht die Gefahr schwerer Verletzungen.



Gefahr der Kontaminierung bei Verwendung von biologischen Gefahrenstoffen. Dieses Symbol weist auf Lebensgefahr, das Risiko schwerer Verletzungen für den Bediener des Gerätes oder Schäden am Gerät selbst hin, die durch Arbeit mit möglicherweise gefährlichen biologischen Substanzen entstehen können.



Weist auf Lebensgefahr oder das Risiko schwerer Verletzungen für den Benutzer des Gerätes aufgrund gefährlicher Stromspannung hin.



Gleichstrom  
Mit diesem Symbol ist auf dem Gerät die Art der Energieversorgung gekennzeichnet: Gleichstrom.



Stromversorgung : AN.



Vor Inbetriebnahme und Betrieb des Geräts ist die Dokumentation zu beachten.

## 1.2 Warn- und Sicherheitshinweise

Der autoMACS Pro Separator ist ein hochmodernes, computergesteuertes Gerät zur automatischen Separation magnetisch markierter Zellen mittels MACS-Technologie. Der MACS MiniSampler ist an den autoMACS Pro Separator angeschlossen und damit Teil der Zellseparationsvorrichtung. autoMACS Pro Separator und MACS MiniSampler gelten bei Einbau und Bedienung durch fachkundiges Personal und Beachtung der allgemein üblichen Sicherheitspraktiken und Hinweise in dieser Bedienungsanleitung als sicher. Dieses Kapitel erklärt die im Umgang mit dem Gerät möglichen Gefahren und gibt wichtige Sicherheitshinweise zur Vermeidung dieser Gefahren. Halten Sie sich genau an diese Hinweise, um sich selbst und das Gerät vor möglichen Gefahren zu schützen und ein sicheres Arbeitsumfeld zu garantieren. Bei Verwendung des Geräts unter Missachtung der Herstelleranweisungen ist die Sicherheit nicht gewährleistet.

**ACHTUNG:** Bitte lesen und befolgen Sie alle in diesem Benutzerhandbuch gegebenen Hinweise zum Betrieb des Gerätes. Beachten Sie auch alle auf dem Display erscheinenden Warnhinweise. Bewahren Sie dieses Benutzerhandbuch sowie alle weiteren, mit diesem Gerät erhaltenen Sicherheits- und Betriebsanleitungen an einem für alle Nutzer des Geräts zugänglichen Ort auf, so dass diese künftig jederzeit darauf zurückgreifen können.

**ACHTUNG:** Der autoMACS Pro Separator ist ausschließlich ausgelegt für den Betrieb in Innenräumen. Bitte benutzen Sie das Gerät nicht in ausgewiesenen Gefahrenzonen wie etwa sauerstoffangereicherten Arbeitsumgebungen.

Wenden Sie sich an die örtlichen Behörden und Ihren Stromversorger für weitere Informationen zur Stromversorgung, Gebäudeinstallationen, Wartung und Sicherheit für die Installation dieses Gerätes.

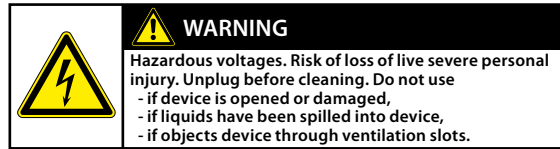
Wenn Sie Sicherheitsbedenken in Bezug auf die Gerätenutzung haben, setzen Sie sich bitte mit Ihrem autorisierten Miltenyi Biotec Service Provider in Verbindung oder kontaktieren den Miltenyi Biotec Customer Support.

## 1.3 Allgemeine Sicherheitshinweise

Um mögliche, mit dem Betrieb des autoMACS Pro Separators verbundene Sicherheitsrisiken zu verringern, beachten Sie bitte die folgenden allgemeinen Sicherheitshinweise. Eine Nichteinhaltung dieser Vorsichtsmaßnahmen könnte Feuer, Gesundheitsschäden, und/oder Schäden am Gerät verursachen.



### 1.3.1 Gefahr des Stromschlags und der Ausbreitung von Feuer



**WARNUNG:** Der Umgang mit elektrischen Geräten birgt das Risiko eines Stromschlags. Um diese Gefahr zu minimieren, öffnen Sie weder das Gehäuse des autoMACS Pro Separators noch anderes Zubehör. Alle Abdeckungen sowie das Geräte-Zubehör dürfen nur von geschultem Personal entfernt werden. Besondere Vorsicht ist geboten beim Umgang mit Flüssigkeiten. Beseitigen Sie ausgetretene oder verschüttete Flüssigkeit sofort. Es darf unter keinen Umständen Flüssigkeit in das Innere des Gerätes eindringen. Ziehen Sie den Netzstecker vor manueller Reinigung des autoMACS Pro Separators.

Eine potentielle Gefahrenquelle liegt auch im Betrieb eines geöffneten, zu Boden gefallen oder beschädigten Gerätes. Ebenfalls sollte unbedingt vermieden werden, dass Flüssigkeit in das Gerät gelangt, Fremdkörper durch die Belüftungsöffnungen eindringen oder von außen in das Instrument hinein gelangen. Bei Auftreten von Flammen oder Rauchentwicklung schalten Sie das Gerät sofort aus, trennen es von der Stromzufuhr und kontaktieren einen autorisierten Miltenyi Biotec Service Provider oder das Miltenyi Biotec Customer-Support -Team. Der Betrieb eines beschädigten Gerätes oder eines Gerätes mit schadhaftem Stromkabel ist ausdrücklich verboten.

### 1.3.2 Starkes Magnetfeld

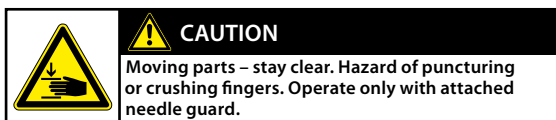


**WARNUNG:** Der autoMACS Pro Separator enthält einen extrem starken Magneten. Halten Sie mit magnetischen Datenträgern (Kreditkarten, Magnetbänder, Speichermedien und dergleichen) und elektronischen Geräten (wie Hörgeräte, Herzschrittmacher, Mess- und Steuergeräte, PCs, Uhren) mindestens 20 cm Abstand zur Magnetabdeckung, da diese durch das Magnetfeld gestört und geschädigt werden können.



Figure 1.1 Warnsymbol für starken Dauermagnet.

### 1.3.3 Quetsch- und Schergefahr



**WARNUNG:** Frontabdeckungen nicht öffnen, wenn das Gerät läuft. Bewegung des Automatikarms und der zugehörigen Komponenten im Betrieb nicht behindern. Finger usw. von allen bewegten Teilen des autoMACS Pro Separator und zugehörigen Komponenten fernhalten, es besteht sonst Gefahr von Quetsch- und Scherverletzungen und Schäden am Gerät. Flüssigkeitspumpen nicht berühren und Leitungen nicht verändern, wenn das Gerät läuft. Gerät immer ausschalten, bevor Arbeiten am Flüssigkeitssystem vorgenommen werden. Laufenden Vorgang immer anhalten oder abbrechen, bevor Arbeiten an Zusatzgeräten wie dem MACS MiniSampler ausgeführt oder Reagenzgläser aus dem Reagenzglasgestell im Sampler entnommen oder dort eingesetzt werden. Sicherheitsmaßnahmen und –vorrichtungen niemals umgehen oder manipulieren.



Figure 1.2 Der Kreis zeigt das Warnsymbol für Quetsch- und Schergefahr.

### 1.3.4 Laserstrahlung

**WARNUNG:** Das Gerät ist zur automatischen Reagenzglasgestellerkennung (Klasse 1M) mit vier oberflächenemittierenden Lasern mit vertikalem Resonator (VSCLs) ausgestattet. Die Strahlung ist nicht sichtbar. Schauen Sie nicht direkt mit optischen Instrumenten (z. B.

Objektiven, Vergrößerungsgläsern und Mikroskopen) hinein. Das Hineinschauen mit optischen Instrumenten in die VSCL-Öffnung innerhalb eines Abstandes von 100 mm kann Ihre Augen schädigen

Das Gerät ist auch mit einem 2D-Code-Leser ausgestattet, der einen sichtbaren Halbleiterlaser als Target-Pointer (Zeiger) zur Einstellung der Leseposition sowie leistungsstarken Leuchtdioden (LEDs) zur Ausleuchtung des Lesefeldes verwendet.

Schauen Sie weder direkt in die Laser- oder LED-Strahlung noch in eine durch eine Spiegelfläche reflektierte Laser- oder LED-Strahlung. Dies kann sonst zu Augenverletzungen führen. Richten Sie den Laserstrahl nicht absichtlich auf andere Personen.

Demontieren, wechseln oder entfernen Sie nicht eingebaute Laser- oder LED-Strahlungsquellen oder deren Halterung. Die Laser- oder LED-Strahlungsquellen hören bei Demontage nicht automatisch auf, Strahlung zu emittieren.

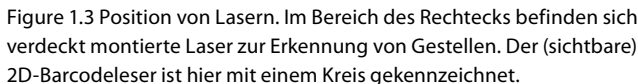
Strahlung von demontierten Geräten kann zu Augenverletzungen führen.

Achten Sie auf den optischen Weg des Laserstrahls oder der Reflexion durch eine Spiegelfläche. Achten Sie bei der Einrichtung des autoMACS Pro Separator darauf, dass sich der optische Weg des Laserstrahls beim Betrieb nicht auf gleicher Höhe mit dem menschlichen Auge befindet.

Vermeiden Sie, dass Wasser, Öl, Staub oder andere Fremdkörper dem Öffnungsfenster des 2D-Code-Lesers anhaften. Dies kann zu Lesefehlern führen. Stellen Sie vor der Reinigung des Scanners sicher, dass keine Laserstrahlen mehr emittiert werden. Der Umgang mit dem Laser kann sonst zu Augenverletzungen führen. Verwenden Sie zum Abwischen von Substanzen auf dem Scanner ein weiches, trockenes Tuch. Verwenden Sie keinen Alkohol oder andere Reinigungssubstanzen.

Der autoMACS Pro Separator ist nach Norm IEC 60825-1: 1993 + A1: 1997 + A2: 2001 als Klasse 1M-Laserprodukt eingestuft.

**WARNUNG:** Die Verwendung von anderen als hierin genannten Bedienungselementen sowie die Anpassung oder Durchführung von anderen als hier genannten Verfahren kann gefährliche Strahlung freisetzen.



In diesem Abschnitt werden die Anforderungen an einen betriebssicheren Standort für Ihren autoMACS Pro Separator beschrieben. Lesen Sie die Anleitungen in diesem Abschnitt und stellen Sie sicher, dass der Gerätestandort entsprechend vorbereitet ist, bevor Sie das Gerät in Betrieb nehmen.

Berücksichtigen Sie bei der Aufstellungsplanung die in diesem Abschnitt beschriebenen Vorkehrungen, um Gerätestörungen zu vermeiden und die Wahrscheinlichkeit umgebungsbedingter Geräteausfälle zu verringern.

**WICHTIG:** Örtliche Arbeitsschutzbestimmungen, Laborrichtlinien, Sicherheitsnormen und Unfallverhütungsvorschriften müssen auf jeden Fall beachtet werden.

### 1.4.1 Montagezubehör

Stellen Sie den autoMACS Pro Separator niemals auf einen instabilen Labortisch, Laborwagen, Untersatz, Laborstativ, oder Laborkonsole. Als Folge davon könnte das Instrument zu Boden fallen, dadurch könnten schwere Verletzungen und/oder Schäden am Gerät verursacht werden. Benutzen Sie daher nur von Miltenyi Biotec empfohlenes oder direkt mit dem Gerät erhältliches Labormobilar. Versuchen Sie nicht, den autoMACS Pro Separator in eine Einbaukonstruktion zu integrieren oder auf begrenztem Raum, z.B. in einem Laborregal, unterzubringen, es sei denn, es wurde eine spezielle Vorrichtung dafür entwickelt, es ist für ausreichende Belüftung gesorgt und die Montageanleitung für das Gerät wurde befolgt.

#### **1.4.2 Belüftung**

Das Instrument sollte nicht in der Nähe von Radiatoren, Heißlüfter, Öfen, oder anderen, Wärme erzeugenden Geräten stehen (Verstärker eingeschlossen). Ermöglichen Sie im Betrieb eine ausreichende Luftzirkulation im Abstand von mindestens 15 cm in allen Richtungen um den autoMACS Pro Separator, um eine ausreichende Kühlung zu gewährleisten. Vermeiden Sie, das Gerät direkter Sonneneinstrahlung auszusetzen. Öffnungen und Schlitze am Gerät sind zur Belüftung gedacht und sollten niemals bedeckt oder blockiert werden, da sie das Gerät vor Überhitzung schützen und sicheren Betrieb ermöglichen. Führen Sie niemals Fremdkörper durch Öffnungen in das Gerät ein.

#### **1.4.3 Wassereintritt und Nässe**

Setzen Sie das Instrument niemals in einer feuchten oder nasskalten Umgebung ein. Setzen Sie das Gerät nie hoher Feuchtigkeit oder Kondensation aus und schützen Sie es gegen Spritzwasser.

#### **1.4.4 Geerdetes Produkt**

Das Gerät ist ausgestattet mit einem Schutzkontaktstecker und einer dreiadrigen Anschlussleitung. Führen Sie den Stecker nicht in eine Steckdose ohne Schutzkontakt ein. Wenn Ihre Steckdose keinen Schutzkontakt aufweist, bitten Sie einen ortsansässigen Elektriker, diese zu ersetzen.

#### **1.4.5 Stromquellen**

Das Gerät sollte nur von einer Stromquelle aus betrieben werden, die den elektrischen Angaben auf dem Typschild entsprechen. Sollten Sie Fragen zur Art der Stromversorgung haben, wenden Sie sich an einen autorisierten Miltenyi Biotec Service Provider oder Ihren lokalen Stromversorger. Benutzen Sie keine Verlängerungskabel oder Steckdosenleiste. Überladen Sie eine Steckdose nicht. Die Gesamtlast darf 80 % der Zweigstromkreisbemessung nicht überschreiten.

#### **1.4.6 Zugänglichkeit der Stromverbindungen**

Der Hauptstromschalter ebenso wie der Netzstecker für das Stromkabel sollten leicht zugänglich sein und sich in möglichst unmittelbarer Nähe zum Bediener des Gerätes befinden. Sollte es erforderlich sein, die Stromzufuhr zu unterbrechen, ziehen Sie den Netzstecker.

#### **1.4.7 Peripheriegeräte**

An die mit „COM“ beschriftete RS-232-Buchse dürfen nur Peripheriegeräte angeschlossen werden, die UL 60950 erfüllen. Die mit „RS232/AUX“ beschriftete Buchse ist nicht belegt. An die mit „External CAN“, „CAN1“ und „CAN2“ beschrifteten Buchsen dürfen nur Original autoMACS Pro-Geräte angeschlossen werden. Die Spannung an diesen Buchsen darf die gefährlichen Werte von 30 Veff und 42,4 V Spitze bzw. 60 V DC nicht übersteigen. An die mit „Bottle Sensor“ beschriftete Buchse darf nur das autoMACS Pro Flaschensen-

sorkabel angeschlossen werden. An die mit „RS232/BCR“ beschriftete Buchse darf nur ein von Miltenyi Biotec empfohlener 2D-Barcodeleser angeschlossen werden. Externe Lasergeräte, die an die mit „RS232/BCR“ beschriftete Buchse angeschlossen werden, müssen IEC 60825-1 erfüllen. Es dürfen nur Anschlusskabel mit einer Länge von max. 3 Metern verwendet werden.

## 1.5 Sicherer Betrieb, Wartung, Transport und Entsorgung

Beachten Sie die folgenden die folgenden Hinweise bezüglich Betriebssicherheit, Wartung, Transport und Entsorgung Ihres autoMACS Pro Separators.

**WICHTIG:** Örtliche Arbeitsschutzbestimmungen, Laborrichtlinien, Sicherheitsnormen und Unfallverhütungsvorschriften müssen auf jeden Fall beachtet werden.

### 1.5.1 Sicherer Betrieb des Gerätes

Falls Ihr Gerät nicht einwandfrei arbeitet und Anzeigen auf dem Display Sie dazu auffordern, den technischen Kundendienst zu kontaktieren, ist die Betriebssicherheit nicht mehr länger gewährleistet. Schalten Sie das Gerät sofort aus, unterbrechen die Stromzufuhr und kontaktieren einen autorisierten Miltenyi Biotec Service Provider oder das Miltenyi Biotec Customer Support Team.

### 1.5.2 Wartung

**WICHTIG:** Versuchen Sie nicht, den autoMACS Pro Separator selbst zu warten oder zu reparieren — es sei denn, es ist in diesem Benutzerhandbuch oder anderen technischen Unterlagen der Miltenyi Biotec GmbH ausdrücklich vermerkt. Wartung und Reparaturen müssen durch geschulte Fachkräfte ausgeführt werden. Falsche oder unsachgemäße Wartung oder Reparatur an Ihrem Gerät kann zur Gefährdung des Anwenders, unvorhersehbaren Resultaten, Fehlfunktionen, Geräteschäden, vorzeitigem Verschleiß und verringerter Lebensdauer führen und kann den Verlust Ihrer Garantieansprüche zur Folge haben.

**Fragen Sie Ihren örtlichen Miltenyi Biotec Vertriebsmitarbeiter nach unseren weit reichenden Vereinbarungen zum Geräteservice und Kundendienst, oder besuchen unsere Website: [www.miltenyibiotec.com/support](http://www.miltenyibiotec.com/support).**

**WICHTIG:** Wenn Ersatzteile benötigt werden, stellen Sie sicher, dass Ihr Service Provider nur Originalteile der Miltenyi Biotec GmbH oder Teile von Drittanbietern verwendet, die von der Miltenyi Biotec GmbH spezifiziert und empfohlen werden. Die Verwendung unautorisierter Ersatzteile kann Fehlfunktionen des Gerätes verursachen und die Ergebnisse von Zellseparationen beeinträchtigen. Die Miltenyi Biotec

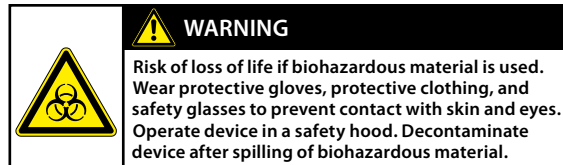
GmbH akzeptiert keinerlei Garantieansprüche und haftet nicht für Fehlfunktionen oder Schäden am Gerät, die auf Verwendung ungeeigneter Verschleiß- oder Ersatzteile zurückzuführen sind. Nach jedweder erfolgter Wartungs- oder Reparaturleistung lassen Sie Ihren autorisierten Miltenyi Biotec Service Provider alle notwendigen Sicherheitsprüfungen durchführen, um sicherzustellen, dass das Gerät sich in vorschriftsmäßigem Zustand befindet.

Nutzen Sie nur von Miltenyi Biotec empfohlenes Zusatzgerät und von uns empfohlene Upgrades zu Ihrem Gerät.

### 1.5.3 Reinigung

Ziehen Sie den Netzstecker vor Reinigung des Gerätes. Verwenden Sie keine Flüssig- oder Sprühreinigungsmittel, sondern nur ein feuchtes Tuch.

### 1.5.4 Gefährliches Material



Wird oder wurde mit biologischen Gefahrenstoffen gearbeitet, muss der Bediener des Gerätes entsprechend den für die verwendeten Substanzen geltenden Warnhinweisen und Schutzbestimmungen eine persönliche Schutzausrüstung tragen. Tragen Sie Schutzhandschuhe, Schutzkleidung, und Schutzbrille, um Berührung der Gefahrenstoffe mit Haut und Augen zu vermeiden. Mangelhafte oder unzureichende Schutzausrüstung kann den Bediener des Instrumentes gefährden. Werden biologische Gefahrenstoffe oder unbekannte Substanzen eingesetzt, sollten Sie mit dem AutoMACS Pro Separator in einer Sterilbank arbeiten. Falls Gefahrstoffe verwendet wurden oder ausgetreten sind, achten Sie auf eine sorgfältige Dekontaminierung des Gerätes.

Säulen, Auffanggefäße und alle weiteren Verbrauchsmaterialien, die in Kontakt mit biologischen Gefahrenstoffen gelangt sind, sollten vor Entsorgung autoklaviert werden. Flüssigabfall sollte autoklaviert oder unter Verwendung eines für das jeweilige spezifische Pathogen geeigneten Desinfektionsmittels dekontaminiert werden, z.B. 10% Bleichmittel, Isopropylalkohol, oder 70% Ethanol.



Figure 1.4 Die Entsorgung der Verbrauchsmaterialien muss gemäß lokal geltender Bestimmungen erfolgen.

### 1.5.5 Transport

Der autoMACS Pro Separator sollte vorsichtig gehandhabt in der von Miltenyi Biotec bereit gestellten Verpackung transportiert werden. Im Gerät können innere Schäden auftreten, falls es großer Erschütterung ausgesetzt oder fallengelassen wird. Sollte wegen Reparatur- oder Wartungsleistungen ein Rücktransport zum Hersteller notwendig werden, dekontaminieren Sie das Gerät vor Versand von jeglichen biologischen Gefahrenstoffen. Wenn Sie Fragen zur vorschriftsmäßigen Dekontaminierung oder zum Versand des Gerätes haben, wenden Sie sich bitte direkt an unseren Technical Support.

### 1.5.5 Geräteentsorgung

Setzen Sie sich bitte direkt mit unserem Technical Support in Verbindung, falls Sie Ihr Gerät entsorgen möchten.





## Leggere prima dell'uso!

**Si prega di leggere tutte le informazioni riportate nel presente manuale d'uso prima dell'utilizzo. La mancata lettura e l'inosservanza delle istruzioni possono condurre ad un impiego improprio o scorretto dello strumento, a manipolazione e manutenzione inadeguate o errate e potrebbero rappresentare un pericolo per l'operatore, avere conseguenze imprevedibili, causare il malfunzionamento o danni allo strumento, condurre ad un'usura prematura e ad una minore durata di esercizio dello strumento, nonché invalidarne la garanzia.**

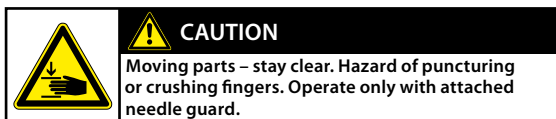
Conservare il presente manuale in un luogo sicuro, accessibile a tutti gli addetti che utilizzano l'unità di separazione autoMACS® Pro (autoMACS® Pro Separator).

Il presente capitolo illustra le istruzioni di sicurezza e i requisiti ambientali per l'installazione dello strumento autoMACS Pro Separator. I seguenti simboli di avvertenza e attenzione servono quale ausilio alla prevenzione di lesioni personali e danni al dispositivo.

## 1.1 Simboli e livelli di pericolo

### Indicazioni di sicurezza

#### Esempio



Le indicazioni di sicurezza informano l'operatore dei rischi potenziali derivanti dal mancato rispetto delle avvertenze e precauzioni descritte di seguito. L'icona sul lato sinistro specifica il tipo di rischio. Il livello di rischio in alto classifica il tipo di pericolo, come indicato in precedenza. Il livello, il tipo e la fonte del pericolo, nonché le possibili conseguenze, i divieti e le misure da adottare vengono indicati come segue.

## Simboli e livelli di pericolo

Di seguito viene presentato un glossario illustrato che descrive i simboli utilizzati nel presente manuale d'uso e sul autoMACS Pro Separator.



Indica una situazione pericolosa che potrebbe comportare lesioni di grado lieve o moderato, qualora non venga evitata.



Indica una situazione pericolosa che potrebbe comportare lesioni gravi o morte, qualora non venga evitata.



Attenzione: consultare il Manuale d'uso per ulteriori istruzioni e procedere con cautela.

Le avvertenze includono il rischio di danni alle attrezzature, gravi lesioni personali o morte.



Pericolo di schiacciamento o di taglio.

Rischio di schiacciamento o di taglio di parti del corpo a causa di componenti meccanici.



Forte campo magnetico



Il campo magnetico può interferire con oggetti magnetizzati e dispositivi elettronici o danneggiare i supporti magnetici per le informazioni. Rischio di lesioni personali gravi per soggetti portatori di pacemaker o impianti medici elettronici.



Rischio di contaminazione in caso di utilizzo di materiale a rischio biologico. Indica il rischio di morte, lesioni gravi a danno dell'operatore o danni alle attrezzature a causa di materiale biologico potenzialmente pericoloso.



Indica il rischio di morte o di lesioni gravi a danno dell'operatore a causa di un livello pericoloso di tensione.



Terminale per conduttore di protezione

Il simbolo è affisso all'interno dello strumento. Avvertenza per il personale di assistenza tecnica



On - acceso (alimentazione)

Off - spento (alimentazione)



E' necessario consultare la documentazione prima di procedere con l'installazione e l'utilizzo dell'apparecchio

## 1.2 Avvertenze e precauzioni

Il separatore autoMACS Pro Separator utilizza tecnologie all'avanguardia. Si tratta di un dispositivo gestito mediante computer per la separazione automatica delle cellule marcate magneticamente mediante la tecnologia MACS. Il campionatore MACS MiniSampler è collegato al separatore autoMACS Pro Separator e rappresenta pertanto un componente del dispositivo di separazione delle cellule. Il separatore autoMACS Pro Separator e il campionatore MACS MiniSampler sono concepiti in modo da garantire un funzionamento sicuro dopo l'installazione, se utilizzati da personale addestrato secondo le norme generali di sicurezza e le istruzioni fornite nel presente manuale d'uso. Le linee guida riportate nella presente sezione illustrano i potenziali rischi associati al funzionamento dello strumento e forniscono importanti informazioni in materia di sicurezza atte a ridurre al minimo detti rischi. Seguendo scrupolosamente le istruzioni, è possibile proteggere se stessi e le attrezzature dai possibili pericoli e garantire un ambiente di lavoro sicuro. Qualora lo strumento venga impiegato in modo non conforme alle istruzioni fornite dal fabbricante, la sicurezza potrebbe risultare compromessa.

**IMPORTANTE:** leggere e seguire tutte le istruzioni operative contenute nel presente manuale e prestare particolare attenzione alle avvertenze indicate sullo strumento. Conservare il presente manuale d'uso e tutte le altre istruzioni operative e di sicurezza fornite insieme alla strumento in un luogo accessibile a tutti gli operatori, per future consultazioni.

**IMPORTANTE:** autoMACS Pro Separator sono destinati unicamente all'impiego interno. Non utilizzare lo strumento in ambienti classificati come luoghi pericolosi, quali gli ambienti con forte concentrazione di ossigeno.

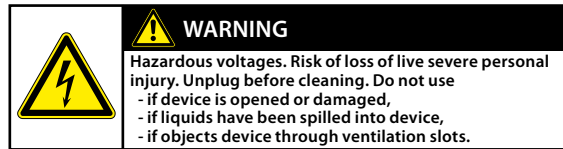
Per ulteriori informazioni relative all'installazione della strumentazione, contattare le autorità locali competenti per la fornitura di energia elettrica, in materia di edilizia, manutenzione o sicurezza.

Qualora abbiate serie preoccupazioni in merito all'impiego sicuro dello strumento, contattare il rivenditore Miltenyi Biotec autorizzato o chiamare il servizio di assistenza clienti Miltenyi Biotec.

## 1.3 Precauzioni generali

Per ridurre i potenziali rischi associati all'utilizzo di autoMACS Pro Separator, osservare le precauzioni generali di seguito elencate. Il mancato rispetto di tali precauzioni può comportare il rischio di incendi, lesioni personali e/o danni allo strumento.

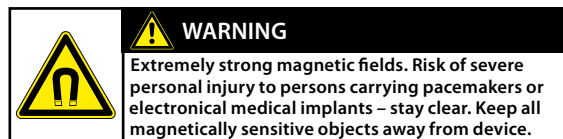
### 1.3.1 Pericolo di scossa elettrica e di incendio



**ATTENZIONE:** I dispositivi elettrici presentano il rischio di scosse elettriche. Per ridurre i rischi di scossa elettrica, non aprire alcun coperchio, tranne i coperchi di accesso anteriore di autoMACS Pro Separator, né altri accessori hardware forniti da Miltenyi Biotec. Tutti gli altri coperchi dello strumento e gli accessori hardware possono essere rimossi esclusivamente da personale autorizzato. Prestare particolare attenzione quando si manipolano liquidi. Pulire immediatamente eventuali schizzi. Non lasciare che i liquidi penetrino all'interno del dispositivo. Staccare il cavo dell'alimentazione prima di pulire manualmente lo strumento autoMACS Pro Separator.

Esistono potenziali rischi in caso di utilizzo di uno strumento AutoMACS Pro Separator aperto, caduto o danneggiato, in caso di infiltrazione di liquidi all'interno dello strumento, in caso di penetrazione di oggetti nello strumento attraverso le fessure di ventilazione o in caso di inserimento accidentale di oggetti nello strumento. In caso di presenza di fiamme o fumo, spegnere immediatamente il autoMACS Pro Separator, staccare lo strumento dalla presa di corrente e contattare un rivenditore Miltenyi Biotec autorizzato o il servizio di assistenza clienti Miltenyi Biotec. È severamente vietato utilizzare uno strumento danneggiato o provvisto di cavo dell'alimentazione danneggiato.

### 1.3.2 Forte campo magnetico



**ATTENZIONE:** autoMACS Pro Separator è provvisto di un magnete estremamente potente. Tenere i supporti magnetici (come carte di credito, nastri magnetici e supporti di memorizzazione) e i dispositivi elettronici (come apparecchi acustici, pacemaker, strumenti di misurazione e controllo, computer e orologi) ad una distanza minima di 20 cm dal coperchio del magnete. Questi oggetti potrebbero essere danneggiati o il loro funzionamento essere compromesso dal campo magnetico.



Figura 1.1 Segnale di avvertenza per forte magnete permanente

### 1.3.3 Pericolo di schiacciamento e di taglio



**ATTENZIONE:** Non aprire i coperchi di accesso anteriore mentre il dispositivo è in funzionamento. Non impedire il movimento del braccio automatizzato e degli accessori hardware durante il funzionamento. Tenere le dita, ecc., lontano da tutte le parti in movimento del dispositivo autoMACS Pro Separator e degli accessori hardware, per evitare lesioni da schiacciamento e ferite da taglio o danni al dispositivo. Non toccare le pompe dei liquidi e non spostare i tubi mentre il dispositivo è in funzione. Spegnerne sempre il dispositivo prima di regolare eventuali parti del sistema della fluidica. Interrompere o sospendere sempre una procedura prima di maneggiare gli accessori hardware, ad es. il campionatore MACS MiniSampler, o prima di caricare/rimuovere le provette dal portaprovette collocato sul campionatore. Non bypassare alcun dispositivo o misura di sicurezza.



Figura 1.2 Il cerchio indica il segnale di avvertenza per il “pericolo di schiacciamento e di taglio”

### 1.3.4 Radiazioni laser

L'apparecchio è dotato di quattro laser a emissione superficiale a cavità verticale (VSCL) per il rilevamento automatizzato (classe 1M). La radiazione non è visibile. Non osservare direttamente il laser con strumenti ottici (p. es. lenti, lenti di ingrandimento e microscopi). Osservando la porta VSCL a una distanza inferiore a 100 mm con uno strumento ottico si potrebbero danneggiare i propri occhi.

L'apparecchio è dotato anche di un lettore di codici 2D dotato di un laser a semiconduttore visibile come puntatore per regolare la posizione di lettura e di potenti diodi luminosi (LED) per illuminare la zona di lettura.

Non guardare direttamente le radiazioni di diodi laser o LED o le radiazioni di diodi laser o LED riflesse da una superficie a specchio. In caso contrario si potrebbero danneggiare i propri occhi. Non rivolgere intenzionalmente il raggio laser verso altre persone.

Non smontare, modificare o rimuovere le sorgenti di radiazioni laser o LED installate o le loro staffe di montaggio. Le sorgenti di radiazioni laser o LED non cessano automaticamente di emettere radiazioni una volta smontate.

Le radiazioni di unità smontate possono essere causa di lesioni agli occhi.

Fare attenzione al percorso del raggio laser o a riflessi da una superficie a specchio. Durante l'installazione del separatore autoMACS Pro accertarsi che il percorso del raggio laser non si trovi alla stessa altezza degli occhi di chi esegue il lavoro.

Evitare che acqua, grasso, polvere o altre sostanze estranee si depositino sulla finestra di lettura del lettore di codici a barre 2D. Ciò potrebbe comportare errori di lettura. Accertarsi di aver arrestato l'emissione laser prima di pulire il lettore. Un'esposizione al raggio laser potrebbe danneggiare i propri occhi. Utilizzare un panno morbido e asciutto per rimuovere qualsiasi sostanza dal lettore di codici a barre. Non utilizzare alcol o altre sostanze detergenti.

Il separatore autoMACS Pro è classificato come prodotto laser della classe 1M secondo lo standard IEC 60825-1: 1993 + A1: 1997 + A2: 2001.

**ATTENZIONE:** L'utilizzo di comandi o regolazioni o l'esecuzione di procedure diverse da quanto qui specificato può essere causa di esposizione a radiazioni pericolose.

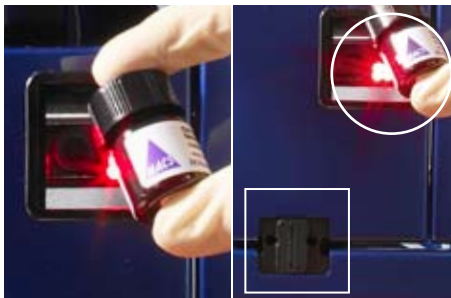


Figura 1.3 Posizione dei laser.

Laser invisibili per il rilevamento dei portacampioni/portareagenti sono collocati nell'area indicata dal rettangolo. Il lettore per codice 2D (visibile) è collocato all'interno del cerchio



## 1.4 Installazione sicura

Questa sezione descrive i requisiti che deve soddisfare il luogo in cui viene collocato il autoMACS Pro Separator onde garantirne un'installazione sicura e il funzionamento corretto. Leggere le istruzioni fornite nella presente sezione e accertarsi che il luogo prescelto sia adeguatamente predisposto, prima di collegare lo strumento alla presa di alimentazione.

Nel predisporre l'ambiente di installazione e nell'organizzare la disposizione delle attrezzature, tenere in considerazione le precauzioni descritte nella presente sezione, in modo da evitare guasti allo strumento e ridurre la possibilità di spegnimenti causati dall'ambiente.

**IMPORTANTE:** vanno sempre osservate le istruzioni di sicurezza sul luogo di lavoro, le procedure di laboratorio e le norme relative alla salute e alla sicurezza del laboratorio, nonché alla prevenzione degli infortuni.

### 1.4.1 Accessori di montaggio

Non collocare il autoMACS Pro Separator su un piano, un carrello, un supporto, un treppiede o una staffa poco stabile, che potrebbe provocare la caduta dello strumento, con la possibile conseguenza di lesioni personali gravi o di gravi danni allo strumento. Utilizzare unicamente piani, carrelli, supporti, treppiedi o staffe raccomandati da Miltenyi Biotec o venduti insieme allo strumento. Non collocare il autoMACS Pro Separator su sistemi incassati o in spazi angusti, come uno scaffale, a meno che non siano stati concepiti specificamente



per accogliere lo strumento e a condizione che vi sia una ventilazione adeguata e che siano state seguite le istruzioni di montaggio dello strumento.

#### **1.4.2 Circolazione dell'aria**

Lo strumento non deve essere collocato in prossimità di radiatori, stufe o altri dispositivi (inclusi gli amplificatori) che producono calore. Accertarsi che via sia un'adeguata circolazione d'aria attorno al autoMACS Pro Separator, mantenendo uno spazio libero di almeno 15 centimetri su ogni lato durante il funzionamento, onde assicurare un adeguato raffreddamento dello strumento. Evitare l'esposizione diretta ai raggi solari. Le fessure e le aperture dello strumento sono necessarie per la ventilazione e non devono essere in alcun caso ostruite o coperte, poiché garantiscono il funzionamento corretto del autoMACS Pro Separator e proteggono il dispositivo dal surriscaldamento. Non inserire mai corpi estranei nelle aperture dello strumento.

#### **1.4.3 Acqua e umidità**

Non utilizzare lo strumento in ambienti bagnati o umidi. Evitare ambienti ad elevata umidità o condizioni di condensa e proteggere lo strumento dagli schizzi d'acqua.

#### **1.4.4 Prodotto con messa terra**

Lo strumento è dotato di un sistema di alimentazione a tre fili ed è provvisto di spina con un terzo polo per la messa a terra. La spina può essere inserita unicamente in una presa di corrente con messa a terra, per ragioni di sicurezza. Non cercare di inserire la spina in una presa sprovvista di messa a terra. Qualora non riusciate ad inserire la spina nella presa, vi consigliamo di contattare il vostro elettricista di fiducia per sostituire la presa.

#### **1.4.5 Fonti di alimentazione**

Lo strumento deve essere alimentato unicamente dalla fonte indicata sulla targa relativa alle caratteristiche di alimentazione del prodotto. In caso di domande sul tipo di alimentazione da usare, contattare il rivenditore Miltenyi Biotec autorizzato o la società elettrica locale. Non utilizzare prolunghe o ciabatte multi-presa. Non sovraccaricare la presa di corrente. Il carico complessivo del sistema non deve superare l'80% della potenza del circuito.

#### **1.4.6 Accessibilità**

Accertarsi che l'interruttore principale e l'attacco del cavo dell'alimentazione siano facilmente accessibili e posti quanto più vicino possibile all'operatore dello strumento. Qualora si renda necessario interrompere l'alimentazione, staccare il cavo dalla presa di corrente.

#### **1.4.7 Periferiche**

Soltanto le periferiche conformi a UL 60950 possono essere collegate al connettore RS232 contrassegnato con "COM". Il connettore

contrassegnato come "RS232/AUX" non è in uso. È inoltre possibile collegare soltanto apparecchi autoMACS Pro originali ai connettori contrassegnati con "External CAN", "CAN1" e "CAN2". I livelli di tensione su questi connettori non devono superare i livelli di tensione ammessi pari a 30 V rms. e 42,4 V di picco o 60 V cc. Al connettore del "sensore flacone" può essere esclusivamente collegato il cavo del sensore del flacone autoMACS Pro. Al connettore "RS232/BCR" può essere collegato soltanto un lettore per codice 2D raccomandato da Miltenyi Biotec. I dispositivi laser esterni collegati al connettore contrassegnato come "RS232/BCR" devono essere conformi alla norma IEC 60825-1. Utilizzare unicamente cavi di connessione inferiori ai 3 metri di lunghezza.

## 1.5 Funzionamento, manutenzione, trasporto e smaltimento sicuri

Seguire le istruzioni di seguito riportate onde assicurare che il funzionamento, la manutenzione, il trasporto e lo smaltimento del vostro autoMACS Pro Separator avvengano in modo corretto e sicuro.

**IMPORTANTE:** vanno sempre osservate le istruzioni di sicurezza sul luogo di lavoro, le procedure di laboratorio e le norme relative alla salute e alla sicurezza del laboratorio, nonché alla prevenzione degli infortuni.

### 1.5.1 Funzionamento sicuro

Qualora lo strumento non funzioni correttamente e le istruzioni o i messaggi visualizzati sullo schermo invitino a contattare l'assistenza tecnica, la sicurezza di impiego non è più garantita. Spegnerne immediatamente il autoMACS Pro Separator, staccare lo strumento dalla presa dell'alimentazione e contattare un rivenditore Miltenyi Biotec autorizzato o il servizio di assistenza clienti Miltenyi Biotec.

### 1.5.2 Manutenzione

**IMPORTANTE:** se non diversamente indicato nel presente manuale d'uso o in altri documenti forniti da Miltenyi Biotec, non eseguire autonomamente la manutenzione del vostro autoMACS Pro Separator. Gli interventi di manutenzione e riparazione devono essere eseguiti da personale qualificato. Interventi di manutenzione e riparazione scorretti o impropri del autoMACS Pro Separator possono rappresentare un pericolo per l'incolumità degli operatori, avere conseguenze imprevedibili, causare malfunzionamenti o danni, provocare l'usura prematura e una minore durata di esercizio dello strumento, nonché invalidare la garanzia.

**Per i contratti di assistenza e manutenzione offerti da Miltenyi Biotec contattare il rappresentante Miltenyi Biotec locale o consultare il sito [www.miltenyibiotec.com/support](http://www.miltenyibiotec.com/support).**

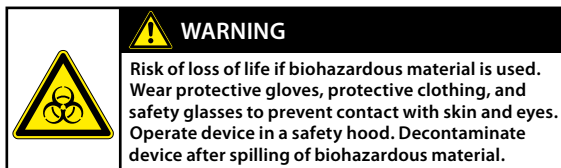
**IMPORTANTE:** qualora si rendano necessari la sostituzione o l'impiego di pezzi di ricambio, accertarsi che il tecnico utilizzi unicamente pezzi di ricambio originali Miltenyi Biotec o ricambi di altri fabbricanti specificati e raccomandati da Miltenyi Biotec. L'impiego di pezzi di ricambio non autorizzati può causare il malfunzionamento del dispositivo e compromettere il risultato della separazione cellulare. Miltenyi Biotec non fornisce alcuna garanzia né si assume la responsabilità per eventuali guasti o danni derivanti dall'impiego di pezzi di ricambio inappropriati. Al termine dell'intervento di assistenza o di riparazione, chiedere al tecnico Miltenyi Biotec autorizzato di eseguire tutti i controlli di sicurezza previsti dalla procedura di riparazione, onde assicurarsi che lo strumento funzioni correttamente.

Utilizzare esclusivamente le funzioni opzionali e gli aggiornamenti raccomandati da Miltenyi Biotec.

### 1.5.3 Pulizia

Staccare il autoMACS Pro Separator dalla presa di corrente prima di eseguire la pulizia. Non utilizzare detergenti liquidi o aerosol; utilizzare sempre un panno umido.

### 1.5.4 Materiale pericoloso



Qualora si utilizzi o sia stato usato del materiale a rischio biologico, l'operatore deve indossare dispositivi di protezione personale conformi alle avvertenze e alle precauzioni relative alle sostanze impiegate. Indossare guanti protettivi, indumenti e occhiali di protezione per prevenire il contatto con la pelle e gli occhi. L'impiego di dispositivi di protezione difettosi o inadeguati rappresenta un rischio per l'incolumità dell'operatore. Lo strumento autoMACS Pro Separator va utilizzato con uno schermo protettivo in caso di trattamento di materiali sconosciuti o pericolosi. Qualora sia stato usato o versato del materiale pericoloso, il sistema deve essere accuratamente decontaminato.

Le colonne, le piastre, le provette e tutti gli altri materiali di consumo entrati in contatto con campioni a rischio biologico vanno sterilizzati in autoclave prima di essere smaltiti. I rifiuti liquidi vanno autoclavati o decontaminati utilizzando un disinfettante idoneo per il patogeno

specifico, ed es. candeggina al 10%, alcol isopropilico o etanolo al 70%.

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Figura 1.4 Lo smaltimento dei rifiuti deve avvenire in conformità alle disposizioni locali vigenti.

### 1.5.5 Trasporto

Lo strumento autoMACS Pro Separator va trasportato con cautela in imballaggi specificati da Miltenyi Biotec. In caso di cadute o di vibrazioni eccessive, lo strumento può subire dei danni interni. Qualora si renda necessario rispedire lo strumento al produttore per interventi di manutenzione, decontaminare lo strumento da eventuali materiali pericolosi prima della spedizione. In caso di domande circa la decontaminazione o il trasporto adeguati, contattare l'assistenza tecnica.

### 1.5.6 Smaltimento

Contattare il servizio di assistenza tecnica per lo smaltimento dello strumento.

### 1.5.7 Compatibilità elettromagnetica

Soltanto per gli USA. Eventuali cambiamenti o modifiche dello strumento non approvati espressamente da Miltenyi Biotec possono inficiare il diritto all'uso dello strumento in conformità all'art. 15 47 CFR.



**Lea la siguiente información antes de usar el instrumento/equipo**

**Por favor, lea íntegramente la información contenida en este manual de usuario antes de utilizar el instrumento/equipo. La lectura y/o seguimiento incorrectos de estas directrices pueden llevar a un uso, manejo o mantenimiento incorrectos del equipo, lo cual puede poner en peligro a sus usuarios, producir resultados impredecibles, derivar en un mal funcionamiento del aparato o que éste sufra daños así como causar un desgaste prematuro y reducir el tiempo de vida del instrumento, pudiendo anular su garantía.**

Mantenga este manual de usuario en un lugar seguro, al alcance de todo aquel que esté usando la Unidad de Separación autoMACS® Pro (separador autoMACS Pro).

El presente capítulo describe las instrucciones de seguridad y los requisitos de emplazamiento de su separador autoMACS Pro. Las siguientes advertencias y precauciones tienen como objetivo evitar daños personales o materiales.

## 1.1 Símbolos y niveles de peligro

### Disposición de advertencias de seguridad

#### Ejemplo



Las advertencias de seguridad informan al usuario sobre riesgos potenciales en caso de que no se sigan las advertencias y precauciones resumidas a continuación. El símbolo que aparece a la izquierda especifica el riesgo de que se trate. El nivel de peligro que encabeza la advertencia clasifica el peligro de que se trate tal y como se mencionó más arriba. El nivel, tipo y fuente del peligro así como las posibles consecuencias, prohibiciones y remedios se indican tal y como aparece a continuación.

## Símbolos y niveles de peligro

El siguiente recuadro recoge un glosario ilustrado que describe los símbolos usados en el presente manual de usuario y en el separador autoMACS Pro.



Indica una situación de peligro que si no se impide puede producir lesiones leves o moderadas.



Indica una situación de peligro que si no se impide puede producir la muerte o lesiones graves.



Atención, consulte el manual de usuario para obtener más información y proceda con precaución. Las advertencias incluyen el riesgo de dañar el equipo, las lesiones personales graves o la muerte.



Peligro de aplastamiento o corte. Riesgo de que se aplaste o corte alguna parte del cuerpo debido a peligros mecánicos.



Radiación láser  
Riesgo de graves lesiones oculares y de piel.



Campo magnético intenso  
El campo magnético puede interferir en objetos magnetizables e instrumentos electrónicos o dañar portadores magnéticos de información. Riesgo de producir lesiones personales graves a personas que lleven un marcapasos o implantes médicos electrónicos.



Riesgo de contaminación si se usan sustancias peligrosas biológicamente. Indica el riesgo de muerte, lesiones graves al operario del aparato o daños al equipo debido a sustancias biológicas potencialmente peligrosas.



Indica el riesgo de muerte o lesiones graves al operario del aparato debido a un voltaje peligroso.



Corriente continua  
El equipo está marcado con el tipo de suministro: corriente continua.



Encendido (suministro)



Apagado (suministro)



La documentación del equipo debe ser consultada antes de proceder con la instalación y operación del sistema

## 1.2 Advertencias y precauciones

El separador autoMACS Pro emplea tecnología puntera. Se trata de un dispositivo controlado por ordenador desarrollado para la separación de células magnéticamente rotuladas con la tecnología MACS. El MACS MiniSampler conecta el separador autoMACS Pro y forma de esta manera parte del dispositivo de separación de célula. El separador autoMACS Pro y el MiniSampler están diseñados para funcionar con seguridad una vez instalados y siempre que sean manejados por personal cualificado de conformidad con las normas generales de seguridad y las instrucciones contenidas en este manual del usuario. Las directrices del presente capítulo explican los potenciales riesgos asociados al manejo del instrumento y suministran información importante a fin de reducir dichos riesgos al mínimo. Si sigue cuidadosamente las instrucciones, se protegerá a sí mismo y al equipo de posibles peligros y creará un ambiente de trabajo seguro. Si este instrumento es manejado de un modo no previsto por su fabricante la seguridad se verá mermada.

**IMPORTANTE:** Por favor, lea y siga todas las instrucciones de uso recogidas en el presente manual de usuario y preste atención a todas las advertencias que aparecen en el instrumento. Guarde para futuras consultas este manual y todas las demás instrucciones de seguridad o de funcionamiento recibidas junto con el instrumento en un lugar accesible para todos sus usuarios.

**IMPORTANTE:** El separador autoMACS Pro está diseñado para ser usado exclusivamente en interior. No utilice el equipo en áreas clasificadas como peligrosas como ambientes con alta concentración de oxígeno.

Póngase en contacto con la autoridad local competente para el suministro de electricidad, construcción de edificios, mantenimiento o seguridad para obtener más información sobre la instalación del equipo.

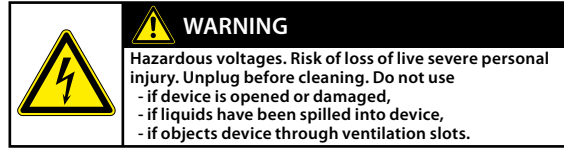
Si tiene serias dudas sobre el manejo seguro del equipo, póngase en contacto con su proveedor de servicios de Miltenyi Biotec autorizado o llame al servicio de atención al cliente de Miltenyi Biotec.

## 1.3 Precauciones generales

Para reducir los potenciales riesgos asociados al manejo del separador autoMACS Pro, por favor observe las siguientes precauciones generales. En caso de no seguir estas precauciones, puede producirse un incendio o causar daños corporales o al equipo.



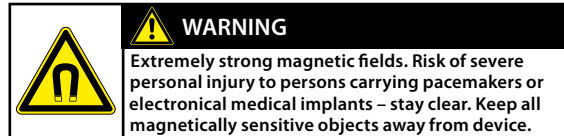
### 1.3.1 Peligro de descarga eléctrica y propagación de fuego



**WARNING:** Los aparatos eléctricos pueden producir una descarga eléctrica. Para reducir este riesgo, no abra ninguna cubierta ni abra tampoco ningún otro accesorio de hardware suministrado por Miltenyi Biotec. Cualquier cubierta así como hardware accesorios deberán ser retirados únicamente por personal autorizado. Se debe tener especial cuidado cuando se manejen líquidos. Limpie inmediatamente los líquidos vertidos. Impida que éstos accedan al interior del aparato. Desenchufe el cable de electricidad antes de proceder a limpiar manualmente el separador autoMACS Pro.

Existe un riesgo potencial en caso de usar separador autoMACS Pro abierto, que se haya caído o que esté averiado, si se han derramado líquidos en el equipo, si se ha colado algún objeto por las ranuras de ventilación o si ha caído algo dentro del equipo. Si salen llamas o humo, apague inmediatamente el separador autoMACS Pro, desenchúfelo y póngase en contacto con un proveedor de servicios de Miltenyi Biotec autorizado o con el servicio técnico de Miltenyi Biotec. Está expresamente prohibido utilizar un instrumento estropeado o cuyo cable de corriente esté dañado.

### 1.3.2 Campo magnético intenso

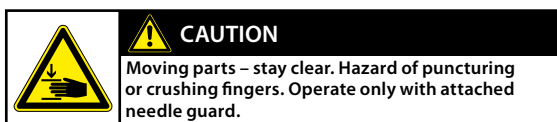


**WARNING:** El separador autoMACS Pro Separator está equipado con un imán extremadamente potente. Mantenga todos los portadores de información magnética (tarjetas de crédito, cintas magnéticas y medios de almacenamiento) y todos los equipos electrónicos (audífonos, marcapasos, instrumentos de medición y control, ordenadores y relojes) a una distancia mínima de 20 cm de la cubierta magnética. El campo magnético puede alterar o dañar dichos objetos.



Figura 1.1 Señal de advertencia para imán permanente potente.

### 1.3.3 Peligro de aplastamiento y corte



**WARNING:** No abrir las cubiertas de acceso frontal durante el servicio del dispositivo. No obstruir el movimiento del brazo automatizado y de los accesorios durante la operación. Mantener los dedos etc. lejos de todas las piezas móviles del separador autoMACS Pro y de los accesorios, para evitar aplastamientos, lesiones de corte o daños en el dispositivo. No tocar las bombas de fluido ni ajustar la tubería durante la operación del dispositivo. Desactivar siempre el dispositivo antes de ajustar cualquier parte del sistema de fluidos. El procedimiento debe pararse o interrumpirse antes de maniobrar los accesorios, p. ej. el MACS MiniSampler, o antes de colocar o retirar los tubos del soporte de tubo colocado en el sampler. No omitir ninguna de las medidas o los dispositivos de seguridad.



Figura 1.2 El círculo abierto muestra la señal de advertencia para el peligro de aplastamiento o corte.

### 1.3.4 Radiación láser

**WARNING:** El dispositivo está equipado de cuatro unidades láser de emisión superficial de cavidad vertical (VSCLs) para la detección automatizada de reactivos (clase 1M). La radiación no es visible. No

mire directamente con instrumentos ópticos (por ejemplo, gafas, lupas y microscopios). Puede ser peligroso para el ojo mirar el puerto VSCL con instrumentos ópticos a una distancia de 100 milímetros.

El dispositivo también está equipado de un lector de código 2D que utiliza un láser semiconductor visible como indicador de blanco para ajustar la posición de lectura y diodos electroluminosos de gran alcance (LED) para iluminar el área de la lectura.

No mirar directamente el láser, radiación LED, láser reflejado o radiación del LED de una superficie reflejada. De otra manera, podría ocasionarse una lesión ocular. No dirigir intencionalmente el rayo láser a otras personas.

No desmontar, modificar ni quitar el láser o la fuente de radiación LED o sus soportes de montaje. El láser o las fuentes de radiación LED no interrumpen la emisión cuando están desmontados.

La radiación de unidades desmontadas puede ocasionar lesiones oculares.

Tener cuidado con la trayectoria del rayo o la reflexión láser de una superficie reflejada. Durante la instalación del autoMACS Pro Separator, tener cuidado de que durante la operación la trayectoria del rayo láser no esté a la misma altura que el ojo humano.

No permitir que agua, aceite, polvo u otras sustancias ajenas se peguen a la ventana de abertura del lector 2D Code. Esto puede causar errores de lectura. Asegúrese de parar la emisión del láser antes de limpiar el escáner. De no ser así, la exposición al láser puede ocasionar lesiones oculares. Utilizar un paño suave y seco para limpiar cualquier sustancia del escáner. No utilizar alcohol u otra sustancia de limpieza.

El autoMACS Pro Separator está clasificado como producto láser 1M de estándar IEC 60825-1: 1993 + A1: 1997 + A2: 2001.

**ATENCIÓN:** El uso de controles, el ajuste o la realización de procedimientos con excepción de los aquí especificados puede dar lugar a una peligrosa exposición de radiación.



## 1.4 Instalación segura

El presente capítulo describe los requisitos que debe reunir el lugar donde desea instalar y operar el separador autoMACS Pro. Lea las instrucciones de este capítulo y asegúrese de que el emplazamiento está correctamente preparado antes de conectar el instrumento a la corriente eléctrica.

Cuando planee la distribución del emplazamiento y la ubicación del equipo tenga en cuenta las precauciones recogidas en este capítulo para evitar averías del instrumento y reducir la posibilidad del cierre de la empresa por razones medioambientales.

**IMPORTANTE:** En todo momento se debe observar la normativa nacional de seguridad en el trabajo, las normas del laboratorio, y los estándares de salud y seguridad en el laboratorio y de prevención de accidentes.

### 1.4.1 Accesorios de montaje

No coloque el separador autoMACS Pro sobre una mesa, carrito, estante, trípode o soporte inestable puesto que podría caerse, causando lesiones corporales graves y/o daños graves al equipo. Utilice exclusivamente una mesa, carrito, estante, trípode o soporte recomendado por Miltenyi Biotec o vendido junto con el instrumento. No coloque el separador autoMACS Pro en un mueble empotrado o un espacio reducido como por ejemplo una estantería, a menos que el mismo haya sido diseñado específicamente para dar cabida al instrumento, que provea una ventilación adecuada y que se hayan seguido las instrucciones de montaje del equipo.

### 1.4.2 Circulación de aire

El instrumento no debería ser colocado cerca de radiadores, rejillas de calor, estufas o cualquier otra pieza de equipamiento (incluidos

los amplificadores) que produzca calor. Permita que circule suficiente aire alrededor del separador autoMACS Pro -deje al menos 15 cm de separación en todas direcciones- mientras está en funcionamiento para garantizar que el instrumento se enfríe adecuadamente. Evite la exposición directa a la luz solar. Las ranuras y aberturas del instrumento sirven para que se ventile y no deben ser bloqueadas o cubiertas, puesto que contribuyen a un funcionamiento seguro del separador autoMACS Pro, evitando que se recaliente. No introduzca ningún cuerpo extraño por las aberturas del instrumento.

#### **1.4.3 Agua y humedad**

No use el instrumento en un lugar húmedo. Evite la elevada humedad o la condensación y proteja a la máquina de salpicaduras.

#### **1.4.4 Producto conectado a tierra**

El instrumento está equipado con un tipo de enchufe de tres varillas que tiene un tercer contacto para toma de tierra. Este enchufe sólo encaja en una toma conectada a tierra. Se trata de una medida de seguridad. No intente enchufarlo en una toma de electricidad no conectada a tierra. Si el enchufe no encaja, póngase en contacto con un electricista para que reemplace la toma de corriente.

#### **1.4.5 Fuentes eléctricas**

Sólo se debería maniobrar el instrumento utilizando la fuente eléctrica indicada en la etiqueta con los valores eléctricos del producto. Si tiene alguna pregunta sobre qué tipo de fuente eléctrica utilizar, póngase en contacto con su proveedor de servicios de Miltenyi Biotec autorizado o con su compañía eléctrica. No utilice alargadores o regletas. No sobrecargue la toma de corriente. La carga total del sistema no debe sobrepasar el 80% de la rama del circuito.

#### **1.4.6 Accesibilidad**

Asegúrese de poder acceder fácilmente tanto al interruptor principal como al conector para el cable de corriente eléctrica y de que éstos estén situados tan cerca del operario como sea posible. Si es necesario desconectar el suministro eléctrico, desenchufe el cable de la toma de corriente.

#### **1.4.7 Aparatos periféricos**

Solamente los dispositivos periféricos que cumplen con la UL 60950 pueden ser conectados con el conector RS232 rotulado con "COM". No se utiliza el conector rotulado con "RS232/AUX". Adicionalmente deben conectarse exclusivamente equipos originales de autoMACS Pro con los conectores rotulados con "External CAN", "CAN1" y "CAN2". Los niveles de voltaje de estos conectores no debe sobrepasar los niveles de voltaje peligroso de 30 V CA y 42.4 V punta o 60 V CC. Se debe conectar solamente el cable de sensor de botella MACS Pro con el conector del sensor de botella. Con el conector "RS232/BCR" debe conectarse solamente un lector de código 2D recomendado por Miltenyi Biotec. Los dispositivos de láser externos conecta-

dos con el conector rotulado con “RS232/BCR” deben cumplir con la norma IEC 60825-1. Utilice exclusivamente cables de conexión con un largo menor a 3 m.

## 1.5 Manejo, mantenimiento, transporte y eliminación seguros

Observe las siguientes instrucciones para asegurar un manejo, mantenimiento, transporte y eliminación de su separador autoMACS Pro seguros.

**IMPORTANTE:** En todo momento se debe observar la normativa nacional de seguridad en el trabajo, las normas del laboratorio, y los estándares de salud y seguridad en el laboratorio y de prevención de accidentes.

### 1.5.1 Manejo seguro

Si el instrumento no funciona correctamente y las instrucciones o mensajes que aparecen en la pantalla aconsejan ponerse en contacto con el servicio técnico, el uso del instrumento ya no es seguro. Apague inmediatamente el separador autoMACS Pro, desenchúfelo y póngase en contacto con el proveedor de servicios de Miltenyi Biotec autorizado o con el servicio de atención al cliente de Miltenyi Biotec.

### 1.5.2 Revisión técnica

**IMPORTANTE:** Salvo que el presente manual de usuario u otra documentación de Miltenyi Biotec especifique lo contrario, no revise usted mismo el separador autoMACS Pro. Las revisiones y reparaciones deben ser llevadas a cabo por personal cualificado. Las revisiones y reparaciones del separador autoMACS Pro incorrectamente realizadas pueden poner en peligro a sus usuarios, producir resultados impredecibles, derivar en un mal funcionamiento del aparato o que éste sufra daños así como causar un desgaste prematuro y reducir el tiempo de vida del instrumento pudiendo anular su garantía.

**Pregunte a su representante de Miltenyi Biotec por los contratos de servicios de asistencia técnica de Miltenyi Biotec o consulte [www.miltenyibiotec.com/support](http://www.miltenyibiotec.com/support).**

**IMPORTANTE:** Cuando se necesiten piezas de recambio o de repuesto, asegúrese de que el proveedor de servicios utiliza exclusivamente piezas originales de Miltenyi Biotec o de otros fabricantes especificados y recomendados por Miltenyi Biotec. El uso de piezas de recambio o de repuesto no autorizadas puede producir un mal funcionamiento del aparato y alterar los resultados de la separación celular. Miltenyi Biotec no cubrirá la garantía ni aceptará ninguna responsabilidad por la avería de aparatos o por los daños resultantes del uso de piezas de recambio o de repuesto inapropiadas. Una vez

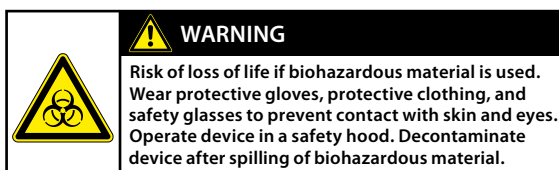
completado el servicio o la reparación, haga que su proveedor de servicios autorizado por Miltenyi Biotec realice todos los controles de seguridad requeridos por el proceso de reparación para asegurarse de que el instrumento está en buenas condiciones de funcionamiento.

Utilice exclusivamente las opciones y actualizaciones recomendadas por Miltenyi Biotec.

### 1.5.3 Limpieza

Desenchufe el separador autoMACS Pro de la toma de corriente antes de proceder a su limpieza. No emplee productos de limpieza líquidos ni aerosoles y use siempre un trapo húmedo.

### 1.5.4 Sustancias peligrosas



Si se utilizan o se han utilizado sustancias biológicamente peligrosas, el operario debería utilizar el equipo de seguridad que aparece en las señales de aviso de las sustancias empleadas. Póngase guantes, ropa y gafas de seguridad para evitar el contacto con la piel y con los ojos. Un equipo de seguridad defectuoso o inadecuado puede poner en peligro al operario. El separador autoMACS Pro deberá ser manejado dentro de una campana de seguridad si se procesan sustancias peligrosas o desconocidas. Si se han utilizado sustancias peligrosas o éstas se han derramado, se debe velar por una desinfección metódica del sistema.

Las columnas, placas, tubos de ensayo y cualquier otro objeto que haya estado en contacto con las muestras peligrosas deberán ser autoclavados antes de poder volver a ser utilizados. Los residuos líquidos deberán ser autoclavados o descontaminados usando un desinfectante industrial apropiado para el patógeno específico, por ejemplo, hipoclorito de sodio al 10%, alcohol isopropílico o etanol al 70%.



Figura 1.4 La eliminación de los residuos debe cumplir la normativa nacional.

### 1.5.5 Transporte

El separador autoMACS Pro debe ser transportado con cuidado en el embalaje especificado por Miltenyi Biotec. Se pueden producir daños internos si es expuesto a vibraciones excesivas o si se cae. En caso de que sea necesario devolver el instrumento a su fabricante para su revisión, límpielo y desinfectelo de cualquier sustancia peligrosa antes de realizar el envío. Si tiene preguntas relativas a la descontaminación o el envío, póngase en contacto con el servicio de asistencia técnica.

### 1.5.6 Eliminación del instrumento/ equipo

Póngase en contacto con el servicio de asistencia técnica si desea desprenderse de su instrumento.

### 1.5.7 Compatibilidad electromagnética

Sólo para EE<sup>UU</sup>. Los cambios y modificaciones del equipo que no estén expresamente aprobados por Miltenyi Biotec pueden invalidar su autorización para operarlo conforme a la parte 15 del título 47 del Código de Regulaciones Federales (CFR).





## 2.1 MACS® Technology—the gold standard in cell separation

MACS® Technology has become the standard method in cell separation. It is based on the use of MACS MicroBeads, MACS Columns, and MACS Separators—strong permanent magnets. In a first step, surface antigens are magnetically labeled in a highly specific manner with monoclonal antibodies coupled to MACS MicroBeads.

After magnetic labeling, the cells are passed over a MACS Column placed in a MACS Separator. Non-labeled cells flow through and can be collected as fractions; labeled cells are retained in the column and can be released after removing the column from the magnet.

Thus, both labeled and non-labeled target cells can efficiently be isolated with MACS Technology. The entire procedure is fast, easy to handle, and gentle to cells, leading to the purification of viable and functionally active cells that can immediately be used for further experiments.

## 2.2 Super-paramagnetic MACS® MicroBeads

MACS® MicroBeads are super-paramagnetic particles of approximately 50 nanometers in diameter, being comparable to the size of a virus. MicroBeads do not change the scatter properties of the cell in the flow cytometer or influence the light-microscopic appearance of the cell. They form a stable colloidal suspension and do not precipitate or aggregate in magnetic fields. MACS MicroBeads are composed of a biodegradable matrix made of iron oxide and polysaccharide. Hence, it is not necessary to detach cell-bound beads after the separation process, saving hands-on time. Usually, MACS MicroBeads do not alter structure, function, or activity status of labeled cells, and they are not known to interfere with subsequent experiments.

The isolated cells can be used directly for subsequent studies or cell culture.

MACS Cell Separation Reagents are highly specific. Thus, it is possible to label, for example, B cells and T cells as well as rare target cells with frequencies as low as  $10^{-8}$ . MACS Technology allows to isolate viable antigen-specific T cells, CD133<sup>+</sup> hematopoietic stem and progenitor

cells, subpopulations of CD34<sup>+</sup> hematopoietic progenitor cells, and disseminated carcinoma cells circulating in the peripheral blood. Finally, MACS MicroBeads offer an extremely flexible tool to isolate many cell types from many species. Several hundreds of reagents for the isolation of human, mouse, rat, and non-human primate cells as well as reagents for indirect labeling of many other cell types are available.

## 2.3 A column-based, high-gradient magnetic cell separation

The MACS Separation Unit consists of a powerful permanent magnet. If a MACS Column is placed into the magnetic field of the Separation Unit, the small ferromagnetic structures of the column matrix disturb this homogenous magnetic field and, thereby, produce high magnetic gradients. In their immediate neighborhood, the ferromagnetic structures generate magnetic forces, allowing to retain target cells labeled with minimal amounts of MACS MicroBeads. Additionally, when the columns are removed from the separator this strong magnetic field is significantly reduced, making the recovery of the retained cells easy and gentle. This method allows separation of viable cells from samples containing a few hundred cells up to 10<sup>11</sup> total cells. Within approximately 15 minutes, the cells are labeled with MACS MicroBeads—nano-sized superparamagnetic particles coupled to specific antibodies. The actual separation of cells over the column is completed within minutes.

## 2.4 The autoMACS® Pro Separator

The autoMACS® Pro Separator is a benchtop magnetic cell sorter that allows gentle sorting of more than 10 million cells per second from a sample of up to 4×10<sup>9</sup> total cells. The instrument is designed for use with any MACS Cell Separation Reagent for research applications. Thus, it is possible to choose between different cell separation strategies according to the respective experimental design—from positive selection of abundant or rare cells to the isolation of untouched cells by depletion of non-target cells. 12 preset separation programs simplify and standardize the application.

**IMPORTANT:** The autoMACS Pro Separator is intended for research applications only and not for diagnostic or therapeutic use.

The autoMACS Pro Separator features automated sample labeling (autolabeling), sample loading, elution of the negative, non-labeled as well as the positive, labeled cell fractions. Up to six samples can be processed in one programming step. Furthermore, automated procedures for maintenance of the system are included. Different wash programs are available to rinse the columns before a new separation is performed. One pair of columns can be used for up to

100 cell separations or for two weeks, whichever comes first. The thin-film transistor (TFT) color touchscreen with intuitive screen menus makes operation and monitoring of the instrument intuitive and easy. Finally, standard MACS Fluid Containers, which are directly attached to the instrument, and ready-to-use sterile MACS Buffers are available for maximum convenience.

The autoMACS Pro Separator is supplied with the MACS MiniSampler and therefore offers an additional sampling option. This new feature allows the sequential processing of multiple samples without further manual handling. The sampler is supplied with three different tube racks and an additional reagent rack. The sampler and tube racks are automatically detected by the autoMACS Pro Separator adding to its operational efficiency.

The user manual aims at explaining the principles of cell sorting with the autoMACS Pro Separator and to assist you by providing step-by-step protocols for cell labeling, separation, and quality control. Tips and hints in the troubleshooting section as well as general protocols and special separation protocols are intended to help optimize your magnetic cell separations.



Figure 2.1: Front image of the autoMACS Pro Separator – the access cover and bottom cover were made transparent for the purpose of illustration.

**autoMACS® Pro Starting Kit contains**

autoMACS Pro Separator, MACS MiniSampler, MACS Reagent Rack 4, 1 MACS Cooling Tube Rack, Chill 5 (for 5 mL tubes), 1 MACS Cooling Tube Rack, Chill 15 (for 15 mL tubes), 1 MACS Cooling Tube Rack, Chill 50 (for 50 mL tubes), Software, Barcode reader, 5x2 autoMACS® Columns, 2x2 column substitutes, autoMACS Pro Buffer Combination, user manual and short instruc- tions, one-year warranty.	130-092-545
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**Technical specifications of the MACS® Cooling Tube Racks and Reagent Racks**

MACS Cooling Tube Racks	Slots	Max. number of samples	Max. sample volume per tube	Max. number of cells per tube
Chill 5	24x5 mL	6 (5 mL tubes)	2.5 mL	5x10 <sup>8</sup>
Chill 15	15x15 mL, 5x5 mL	5 (15 mL tubes)	12.5 mL	2.5x10 <sup>9</sup>
Chill 50	6x50 mL, 3x15 mL, 3x5 mL	3 (50 mL tubes)	50 mL	4x10 <sup>9</sup>
MACS Reagent Rack				
Reagent Rack 4		4 reagent vials		

**Technical specifications of the autoMACS® Pro Separator (the instrument is UL-listed and CE-marked)**

Programs	12 pre-set
Protocol	Single- and double-column enrichment, positive selection, and untouched isolation of target cells
Column capacity	4x10 <sup>9</sup> cells / sample; 2x10 <sup>8</sup> magnetically labeled cells / sample (up to 15 mL of whole blood or bone marrow)
Processing volumes	0.2–50 mL (input) / 0.5–52 mL (output)
Footprint *	605 mm x 353 mm (wxd)
Footprint with MACS MiniSampler *	605 mm x 455 mm (wxd)
Height	392–460 mm (adjustable touchscreen)
Weight	25 kg
Input voltage	100–240 VAC, 50–60 Hz
Power consumption	200 VA

\* Depending on the type of power plug chosen, the depth increases by 27 mm or 62 mm.

The autoMACS Pro Separator is a benchtop instrument that fits in laminar flow hoods or safety cabinets. If the instrument is placed in a laminar flow hood, the following accessories might be required: autoMACS Pro Laminar Hood Plate (# 130-093-246) and, optionally, autoMACS Pro Angle Connector Kit (# 130-093-245). The Laminar Hood Plate provides a stable and even surface, even on potentially bending surfaces. The angle connectors reduce the depth of the instrument to 455 mm (including MACS MiniSampler) for placement in a location with limited space.

The operating environment should be stable and vibration-free, dust-free, sufficiently ventilated, and free from sources of electromagnetic radiation.

Before operating the autoMACS Pro Separator for the first time, carefully read the user manual and contact your local Miltenyi Biotec representative for assistance.

## 3.1 Unpacking and installing the autoMACS® Pro Separator

Read through the following instructions carefully before commencing the installation procedure. Before opening the transportation box, check for any visible external damage to the box. Check also to see if the shock and position indicators (if present) suggest incorrect transportation of the instrument. If there is apparent damage please contact Technical Support for assistance (refer to section 13).

**Note:** The top compartment holds the autoMACS Pro user manual, the short instructions, and various bags containing accessories. Carefully remove these parts.

- 1 Open the box and remove the top compartment of the packaging to reveal the instrument and associated packaging.



Figure 3.1: Top compartment of the autoMACS Pro Separator box.

- 2 Remove boxes containing the MACS MiniSampler and cover, and the MACS Cooling Tube Racks.

Remove the foam packaging from both sides of the autoMACS Pro Separator.

**Note:** Two persons are required to lift the autoMACS Pro Separator. The instrument must be gripped at the base of the orange bottle baskets located at both sides of the instrument. Note that the instrument is heavier at the front. Ensure the front of the instrument is stabilized while lifting it.

Due care must be taken while lifting the autoMACS Pro Separator. Miltenyi Biotec accepts no liability for potential injuries and damages sustained during lifting and/or movement of the instrument.



Figure 3.2: Packing format of the autoMACS Pro Separator and accessories.

- 3 Place the instrument onto a stable worktop surface, e.g., laboratory bench. Remove the plastic bag surrounding the instrument.



Figure 3.3: Positioning the autoMACS Pro Separator securely onto a laboratory bench.

- 4 Note the positions of the MACS MiniSampler guiding (2) and its corresponding slot (1) located at the front of the instrument. Tilt the MiniSampler and slide the guiding into the receiving slot until resistance is met; lower the rack to a horizontal position, i.e., the rack is locked in the position illustrated by the figure below.



Figure 3.4: Location of the MiniSampler guide (2) and receiving slot (1) for the MACS MiniSampler.

- 5 Ensure that the MiniSampler is completely inserted and secure the connection by fastening the MiniSampler screw as shown in the figure below.



Figure 3.5: Securing the MiniSampler by fastening the MiniSampler screw.

- 6 Attach the sensor cables to the cable guide at the back of the autoMACS Pro Separator.



Figure 3.6: Sensor cables are attached to the back of the instrument as shown above.



- 7 Place the MiniSampler cable underneath the autoMACS Pro Separator and connect it to the socket labeled "External CAN" at the rear panel of the instrument. Use the screw driver to fasten the screws of the connector.
- 8 Guide the 2D code reader cable underneath the instrument. Connect the 2D code reader plug with the corresponding outlet at the back of the instrument labeled "RS232/BCR". Use the screw driver to fasten the screws of the connector.
- 9 Attach the sensor cable plug to the socket for sensor cables at the back of the autoMACS Pro Separator labeled "Bottle Sensor" and fasten securely. Use the screw driver to fasten the screws of the connector.

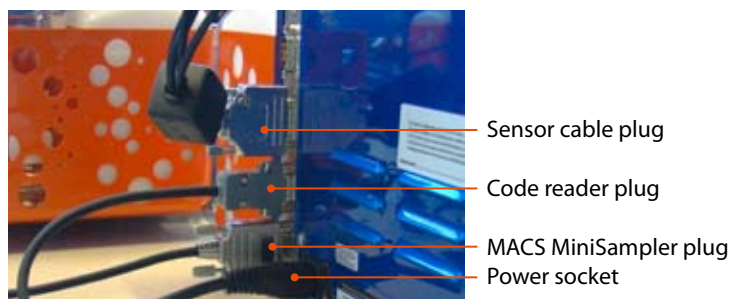


Figure 3.7: Location of the MACS MiniSampler, Sensor Cable, Code Reader, and power sockets at the back of the instrument.

- 10 Carefully remove the uptake port needle from the foam packaging.



Figure 3.8: Close-up of the autoMACS Pro Separator - the needle arm and underside of the touch screen are supported by foam.

**Note:** Ensure that the tubing connected to the uptake port needle can move freely when the needle arm extends, or when the needle moves into the sample uptake position.

- 11 Place the uptake port needle into its guiding at the needle arm.

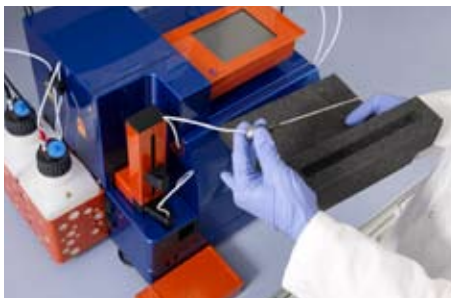


Figure 3.9: The needle port is positioned as illustrated above.

- 13 Note the position of the power socket on the rear panel of the autoMACS Pro Separator (figure 3.7). Ensure that the main power switch is in position "0".
- 14 Connect the power cord.
- 15 Elevate the touchscreen and insert the memory card as shown below.





Figure 3.10: Inserting the software memory card.

- 16** Replace the fluid containers. Refer to section 3.2 for full details.
- 17** Switch on the autoMACS Pro Separator.
- 18** Secure the MACS Reagent Rack 4 onto the MiniSampler into the left recess. The engagement hook has to snap into the undercut.



Figure 3.11: Securing the MACS Reagent Rack 4 onto the MACS MiniSampler.

**Note:** Racks can be pre-cooled for 3–4 hours at 2–8 °C. Do not cool below 0 °C since samples may freeze. If recognition of the tube rack fails, the instrument will display a screen for manual selection of the tube rack. Before confirming the choice, ensure that the rack is placed correctly into the recess – the MACS Triangle should face towards the user.

- 19 Set a cool tube rack (e.g. Chill Rack 5) onto the MiniSampler into the right recess ensuring that the rack barcode is facing the autoMACS Pro Separator.



Figure 3.12: Positioning the Chill Rack 5 adjacent to a MACS Reagent Rack 4 on the MACS MiniSampler.

- 20 Perform a system calibration test: refer to section 3.3 for more details.

## 3.2 Replacing fluid containers and the connection of fluid sensor cables

Operating the autoMACS Pro Separator requires Running Buffer, Washing Solution, and storage solution (absolute ethanol diluted to 70% with double-distilled water). It is recommended to operate the instrument with ready-to-use MACS Buffers. The autoMACS Pro Separator is delivered with four empty fluid containers which are connected to the instrument with specifically designed bottle closures. The bottle closures consist of a fluid uptake port or a fluid outlet port (waste container) as well as a sensor for measuring electrolyte conductivity. The fluid containers, bottle closures, and fluid sensor cables are color-coded for easy handling (refer to table 3.1 below).

Container	Symbol	Container	Symbol
Running Buffer (blue)		Storage solution (black)	
Washing Solution green		Waste (red)	

Table 3.1: Symbols and color-coding of fluid containers.

**Note:** The 70% ethanol does not contain electrolytes. Therefore, the filling status of the ethanol container cannot be determined.

- 1 Install one fluid at a time. Please note the corresponding color-coding (refer to table 3.1).

**Note:** When removing the waste container, unscrew the bottle closure and then fasten a standard lid (i.e. without sensor cables) onto the bottle before removing the waste container from the basket. This is to ensure that the contents of waste container are not accidentally split over the autoMACS Pro Separator during removal from the basket.

**Note:** The correct positioning of each solution container—recognizable by the color code and the symbols—is crucial in order to perform successful procedures using the autoMACS Pro Separator.

To keep buffers sterile, each bottle closure should be equipped with a hydrophobic air filter. Avoid any contact of hydrophobic air filters with fluids as this may cause clogging of the filter.

When working with biohazardous samples, it is recommended to fill the waste container with 100 mL of disinfectant before use (e.g. MACS Bleach Solution, # 130-093-663). For proper disposal, please follow local regulations and carefully read the chapter 1.

- 2 Unscrew bottle closures counter-clockwise and remove the empty container. Do not disconnect the color-coded tubing.
- 3 Do not open a fresh bottle until it is placed in the basket! Place the bottle in its appropriate position, remove the cap, and fasten the bottle closure. Note the color-coding and connect each sensor cable to the respective bottle closure.
- 4 Attach the sensor cable plug to the socket for sensor cables at the back of the autoMACS Pro Separator labeled "Bottle Sensor" and fasten securely.

Connect the hydrophobic air filters (0.2  $\mu\text{m}$ ) to the appropriate connectors on the bottle closures.



### 3.3 Running the autoMACS® Pro Separator for the first time: performing a test calibration

**Note:** Ensure that the autoMACS Pro Separator has been correctly installed (section 3.1) and that the empty fluid containers used for transport purposes have been replaced by fresh fluid containers (section 3.2).

#### 3.3.1 Switch on the autoMACS® Pro Separator

The main power switch is located on the right side of the instrument in front of the container baskets ("I" indicates "on", "O" indicates "off"). Switch on the autoMACS® Pro Separator.



Figure 3.13: Location of the on/off switch.

#### 3.3.2 Check the date and time

- To set the time and date perform the following steps:  
Select **Option**, **User settings**, and **Set\_time**. Select **Run**. Highlight either the **time** or **date** fields by touching the display. Use the keypad to change the date or time accordingly.



Figure 3.14: Select the desired field and use the numeric keypad to modify the date and/or time.

Select **OK** to return to the **Option** menu. The procedure can be cancelled at anytime by selecting **Cancel**.

### 3.3.3 Perform a test calibration

- 1 On the touchscreen click: **Option**, **Calibr\_1**, **Run**, and **Test**.

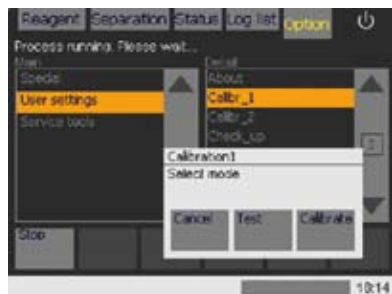


Figure 3.15: Instructing the instrument to perform a test calibration.

#### 2 Test calibration: Chill Rack

Ensure that a Chill Rack 5 is placed on the MACS MiniSampler. Click **Done** to continue test calibration.

#### 3 Test calibration: Reagent Rack

Ensure that a Reagent Rack 4 is placed on the MACS MiniSampler. Click **Done** to continue test calibration.

#### 4 Test calibration: Control position of needle in the washing station

The instrument will automatically position the needle arm in the washing station. If the needle is not correctly positioned, click **Move Up** or **Move Down** to adjust the needle position accordingly. Click **Done** to continue the test calibration sequence.



Figure 3.16: Test calibration of the needle arm position in the washing station.

#### 5 Test calibration: Control position of needle in tube

The instrument will insert the needle arm into tubes located in rack positions A1, B1, C1. Click **Move up** or **Move down** to adjust the position of the needle in the tube. The needle arm should almost touch the bottom of the middle point of a standard 5 mL plastic sample tube. If the needle is correctly positioned, click **Done**.

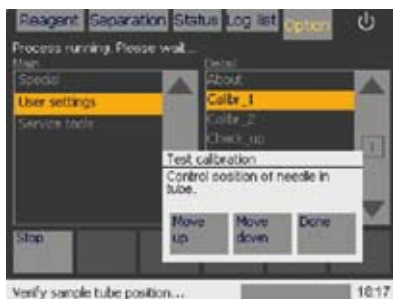


Figure 3.17: Click “Done” if the needle is correctly positioned; click “Move up” and “Move down” to adjust the position.

**Note:** After test completion the instrument will proceed to the next test calibration step: control position of needle in vial.

#### 6 Test calibration: Control position of needle in vial

The instrument will insert the needle arm into the MACS Reagent Rack vial positions R1, R2, R3, and R4. Click **Move up** or **Move down** to adjust the position of the needle in the vial. The needle arm should almost touch the bottom of a MACS Reagent vial. If the needle is correctly positioned, click **Done**.





**Note:** If test calibration 1 fails, perform a calibration 1. Refer to section 12.2 for details.



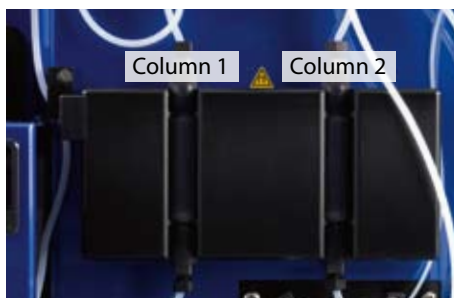
Figure 3.18: Test calibration: Control position of needle in vial.

7 Test calibration is completed.

### 3.3.4 Installation of the autoMACS® Columns

Remove the column substitutes and install the autoMACS® Columns according to the following instructions:

- 1 Open the front door and note the position of the tubing and autoMACS Column slots in the magnet cover (column 1 to the left, column 2 to the right).



- 2 Ensure that the fluid containers are filled with solutions.
- 3 Using the touch screen, select **Option** and **Special** from the “Main” panel.
- 4 Select **Col\_ex** (column exchange) from the “Detail” panel.



Figure 3.20: Selecting column exchange from the “Option” tab.

**Note:** When delivered, the autoMACS Pro fluidic system is filled with double-distilled water.

The autoMACS Columns are reusable for two weeks or up to 100 separations, whichever comes first.

The instrument automatically records the date of the column exchange and can display the due date for the next column replacement if the program Column exchange (Col\_ex) has been used for column installation.

Columns do not have a top or bottom and do not require special orientation in the column holder.

- 5 Select **Run** to start program **Col\_ex**. A popup dialog offers four options:  
**Remove** to remove columns;  
**Install** to remove and install new columns;  
**Sep count** to inform the software how many separations have been performed;  
**Exit** to cancel this process.

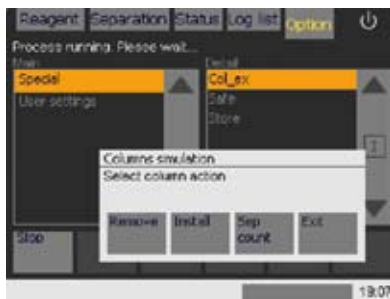


Figure 3.21: Select column action dialog box.

**Note:** Only install one column at a time.

- 6 Select **Install** from the popup dialog box.
- 7 Wait until the instrument prompts you to exchange the autoMACS Columns before proceeding. Select **Done**.
- 8 Using both hands take the top and bottom of the column one substitute and pull gently but firmly to remove it from its slot.



- 9 Place a wide mouth container under the column substitute. Hold the column substitute in one hand and gently unscrew the bottom column connector counter-clockwise. Unscrew the top column connector while holding the column over the container to catch any fluids. Store the column substitute in the autoMACS Pro Starting Kit box.



Figure 3.22: Unscrewing the top column connector for column removal.

**Note:** The program will then proceed to wash the columns with autoMACS Running Buffer. Check that the column is securely fastened to the column connectors and that no buffer is leaking.

- 10 Insert one end of a fresh column into the bottom column connector and gently screw in the column by turning it clockwise until you feel a resistance. Point the column towards the top of the instrument and screw in the top column connector.
- 11 Align the column with the top column connector sitting on the guiding of the magnet cover. Press the column into the slot until you feel the guides click. Verify that the column is placed in the center of the magnet cover.
- 12 Repeat steps 8 through 11 to install the second column.
- 13 Ensure that the tubes are securely fastened to the columns and that the tubing is neither pinched nor obstructed.
- 14 Press **Done**.
- 15 Close the front door. The unit is now ready to perform cell separations.

### 3.3.5 Priming the autoMACS® Pro Separator

Priming implicates the initial cleaning and filling of the autoMACS® Pro Separator tubing system before cell separations are performed. The autoMACS Pro Separator must be primed each time the instrument is switched on.

**Note:** Read the warnings and precautions section before priming the autoMACS Pro Separator for the first time.

- 1 Fill all bottles with the appropriate solutions and empty the waste bottle.
- 2 Switch on the instrument. After initialization is completed, the **Status** menu will be displayed. Verify that the touchscreen symbols for all fluid containers are green. If this is not the case, check if the fluid sensor cables are connected to the correct fluid containers. The symbol for the ethanol container remains gray (fluid level not checked by system). At this point, the bottle illumination is yellow.

**Note:** When working with biohazardous samples, it is recommended to fill the waste container with 100 mL of disinfectant before use (e.g. MACS Bleach Solution). For proper disposal, please follow local regulations and carefully read the chapter 1.

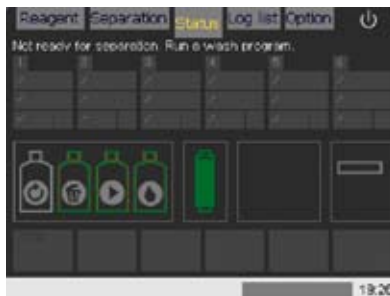


Figure 3.23: The status menu. The fluid level status and column are green for "go". A wash or instrument prime has not yet been performed.

- 3 Select menu **Separation** and **Wash Now** from the lower menu. You now have the option to perform a quick rinse (**Qrinse**) or full rinse (**Rinse**).



Figure 3.24: Select "Rinse" to prime the autoMACS Pro Separator.

- 4 Select **Rinse** and **Run** to start the priming process. The progress will be displayed at the bottom of the touchscreen menu.
- 5 When priming is finished, the instrument will display **Ready for separation** in the **Status** menu. The bottles are illuminated green.



Figure 3.25: The instrument has been primed (rinsed).

### 3.3.6 Checking the fluid levels

The autoMACS Pro Separator automatically ensures that the uptake port is filled with buffer, that fluid containers carry enough fluid for

**Note:** A cell separation template can already be programmed or reloaded during the priming procedure. By selecting Run the user will be asked to confirm that enough running buffer is available; thereafter priming will begin.

The autoMACS Pro Separator will display a warning screen if the buffer supply is low or if the waste bottle is full. If no wash program has been performed before the first separation, a warning screen will ask to rinse the system.

You may interrupt any program by selecting **Stop**. The **Stop** button is located at the bottom left hand corner in all menus.

When priming the instrument for the first time or when the instrument was not serviced for a long period of time, it is recommended to visually inspect the fluidic system for potential leaks. Open the front door after priming the instrument. If there is any sign of leakage (e.g. salt deposits), tighten the respective tubing connection. Close the front door and proceed with the separation.

one separation, and that the waste container can collect fluid from at least one separation. If more than one separation is performed, ensure that the containers contain sufficient fluid for all the separations and that the waste container is empty.

To verify the status of the bottles, select the menu **Status** from the upper navigation bar. On the left hand side of the menu, four symbols display the solution containers and their filling status. If the fluid containers are full and the waste container is empty, the symbols are green. If the solution containers are empty or the waste container is full, the respective symbols are red. The symbol for the ethanol container remains gray. It is recommended to select the menu **Status** when the autoMACS Pro Separator is in operation.

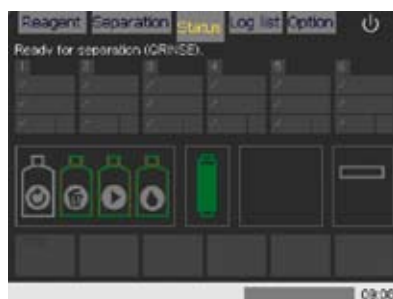


Figure 3.26 Status of the fluid containers is green for ready.

### 3.3.7 Test of barcode scanner

- 1 Select **Reagent**.
- 2 Press **Read Reagent**.
- 3 Place barcode (figure 1.3) in front of barcode scanner



- 4 "CD4 MicroBeads, human" will appear as scanned reagent.



# An overview of the autoMACS® Pro touchscreen user interface

## 4

The autoMACS® Pro Separator is operated through a TFT color touchscreen. After switching on the instrument, the startup screen will appear.



Figure 4.1: The autoMACS Pro Separator startup screen.

## 4.1 Working with the lower menu bar



Figure 4.2: The lower menu bar of the "Separation" menu.

A lower menu bar is accessible from all **Menu** screens. Depending on the status of the instrument and the selected upper tabbed menu option, the lower menu buttons switches between an inactive (grey background) and active state (white background) state. For example, **Stop** can only be selected when the instrument is actively performing a process such as cell separation.



Figure 4.3: Left: Stop button is active and can be selected. Right: Stop button is inactive and cannot be selected.

## 4.2 The main menu screen

Five main menus allow easy interaction with the instrument. They are accessed through the upper tabbed menu:

Tab	Function
Reagent:	To define the position of MACS Reagents on the MACS Reagent Rack 4.
Separation:	To define an autolabeling and/or cell separation strategy for up to six samples. In addition, cell processing procedures can be saved as templates for regular use.
Status:	The instrument status is displayed at a glance.
Log list:	The log list details completed actions and errors.
Option:	Users can perform special procedures such as exchange of MACS Columns, instrument calibration, and service steps.

Table 4.1: Feature overview of the upper tabbed menu.

After instrument initialization the screen automatically displays the **Status** menu, which helps to monitor the instrument during installation and operation. Color-coded symbols indicate the status of the hardware components. Further information on a particular component can be obtained by touching the symbol.



Figure 4.4: The main "root" menu screen displays instrument "Status" at startup.

### 4.3 Status menu

The autoMACS Pro Separator is a sensor-controlled instrument that allows easy monitoring during operation. At a glance the instrument status can be determined by viewing the **Status** menu at any time.

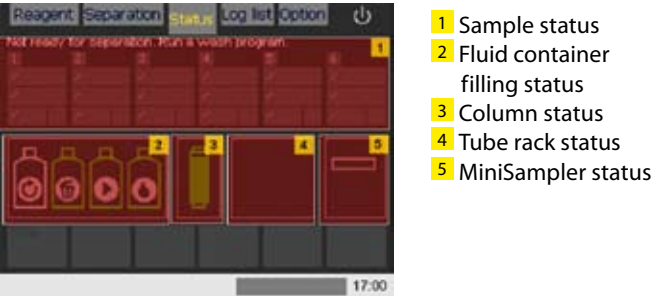


Figure 4.5: Overview of the instrument status panels.

A description of how to monitor the instrument's status using the **Status** menu follows.

### 4.3.1 Status of fluid containers



Figure 4.6: Fluid container status symbols. Left: Fluid containers are ready. Right: Fluid containers need replaced.

The status of fluid containers is indicated by color-coded graphic symbols and by a text table.





Container	Symbol	Symbol color and user action
Running Buffer		Green: no action required Red: refill container Gray: connect bottle sensor
Washing Solution		Gray: connect bottle sensor
Storage solution		Gray: no liquid detection; visually check volume
Waste		Green: no action required Red: empty waste Gray: connect bottle sensor

Table 4.2: Status of fluid containers displayed in the "Status" menu


In addition to color-coded graphic symbols of the fluid containers, a popup text table also reports fluid container status. Touch any fluid bottle symbol to activate the popup textbox. To close the popup table, select . Moreover, the symbols are also red, when the sensor cables are connected to a wrong bottle closure.





Figure 4.7: Fluid container status. By touching any fluid container symbol a popup box displays a text report of the fluid container status.

### 4.3.2 Column status



Figure 4.8: The column status graphic. Left: The column is ready. Right: The column must be changed.

If the column symbol is green, no action is required. If the symbol is red, the columns must be exchanged. The level of the green fill on the column symbol indicates the remaining operation-life of the autoMACS Columns. Touch the column symbol to activate the column status popup textbox.



Figure 4.9: Column status. By touching the column symbol a pop-up box displays a text report of the column status.

### 4.3.3 Tube rack status

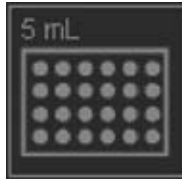


Figure 4.10: Tube rack status graphic: a 5 mL tube rack was detected. If no tube rack is detected no graphic is displayed.

Rack detection occurs prior to starting the separation process. Before cell labeling and/or cell separation is performed the instrument will not attempt to detect the rack. Touch the position of the rack status graphic in order to view the tube rack popup text table.

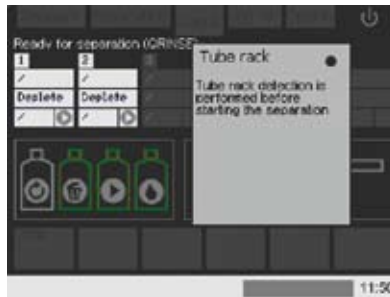


Figure 4.11: Tube rack status text box: No rack is detected until cell separation is performed.

### 4.3.4 MACS® MiniSampler status



Figure 4.12: MACS MiniSampler status graphic. Left: The MiniSampler was successfully installed. Right: No MiniSampler was detected.

If the MACS® MiniSampler has been detected correctly a rectangular symbol is displayed. If it has not been detected a hand symbol will be displayed in the same field.

## 4.4 Separation menu

The **Separation** menu schematically represents a sample rack and allows definition of sample processing strategies for each sample rack position. For each sample rack position it is possible to define cell labeling, cell separation, and washing programs.

A single wash program can be performed by selecting **Wash Now** from the lower navigation bar.



Figure 4.13: The separation menu.

An overview of the separation menu follows:



- 1 Sample rack template
- 2 Sample labeling options
- 3 Sample processing volume
- 4 Separation program
- 5 Wash procedure

### Sample rack template

The positions in the programming field (1) correspond to the sample positions in the tube rack. Positions 1–6 are used in combination with tube rack Chill 5, positions 1–5 with rack Chill 15, and positions 1–3 with rack Chill 50.

- 1 To select/deselect a sample position touch to highlight the desired position 1–6.
- 2 Select/deselect a sample position by touching the display.



Figure 4.14: Sample positions 1, 3, and 4 were selected.

### Sample labeling options

The **Labeling** submenu (2) is used to instruct the instrument:

- if autolabeling is to be performed on a sample.  
The default setting is “/”, which indicates that NO autolabeling will be performed.
- the type of autolabeling that will be performed.  
A list of product options is only visible if reagents have already been assigned to positions on the reagent rack using the **Reagent** menu.



Figure 4.15: “Labeling” submenu. Using the MACS Reagent Rack 4, a total of four reagents may be used at any given time.

### Assigning sample volumes

The **Volume** submenu (3) is used to inform the instrument about the available sample volume. To enter or modify a volume:

- 1 Ensure that the appropriate sample template position is highlighted.
- 2 Select the **Volume** submenu.
- 3 Using the numeric keypad enter the sample volume.  
For autolabeling enter the sample volume for the first labeling step as outlined in the corresponding data sheet (e.g. 160  $\mu\text{L}$  /  $2 \times 10^7$  cells for labeling with CD4<sup>+</sup> MicroBeads, human or 120  $\mu\text{L}$  /  $4 \times 10^7$  cells for labeling with Monocyte Isolation Kit II, human).



Figure 4.16: Entering the sample volume in microliters ( $\mu\text{L}$ ) for Monocyte Isolation Kit II, human.

- 4 Note that the minimum incubation volume after the addition of the first reagents may not be less than 200  $\mu\text{L}$ .
- 5 Select **Enter**.

### Assigning a cell separation program

The **Separation** submenu (4) is used to instruct the autoMACS Pro Separator which cell separation program should be applied to each sample. Several sample positions can be highlighted to assign a separation condition for multiple samples. Refer to section 6.3.1 for a detailed explanation of the various cell separation strategies. To assign or modify a cell separation program:

- 1 Highlight the appropriate sample(s) using the sample template.
- 2 Scroll through the **Separation** submenu using the arrows ▲ / ▼.
- 3 Select and highlight a **Separation** program.



Figure 4.17: Positive cell separation-sensitive mode was selected.

### Assigning a wash program

The **Wash** submenu (5) is used to instruct the autoMACS Pro Separator which wash program should be applied to each sample. Three wash programs are available to choose from:

**Qrinse** (quick rinse) - ●: Recommended to save time between sample separation steps. The tubing and column receive a quick rinse.

**Rinse** - ●: The system is 'primed', receiving an extensive wash. Rinse is recommended between rare cell, whole blood, cord blood, and bone marrow separations.

**Sleep** - : A rinse is performed before the system is shutdown.

To assign or modify a wash program:

- 1 Highlight the appropriate sample(s) using the sample template.
- 2 Scroll through the **Wash** submenu using the arrows ▲ / ▼.
- 3 Select (highlight) a **Wash** program.



Figure 4.18: A rinse program was selected.

### Working with separation templates

For convenience it is possible to load and save separation templates.

**Separation** templates can be used in combination with **Reagent** templates (refer to above).

To save a separation template:

- 1 Configure the sample template, an example follows.



Figure 4.19: A sample template was setup to process two samples.

Sample 1: Isolation of untouched human monocytes using the Monocyte Isolation Kit II, human (# 130-091-153); autolabeling program MO.IK.h; separation program "Deplete".

Sample 2: Direct labeling and positive cell enrichment of CD4<sup>+</sup> cells using CD4 MicroBeads, human (# 130-045-101); autolabeling program CD4.h; separation program "Possel".

- 2 Select **Save Template**.
- 3 Allocate a name to the template. In this example the template was saved as "EXPT\_2A" (experiment 2a).



Figure 4.20: Using the alphanumeric keypad assign a name to the template.

#### 4 Select **Ok**.

To load a reagent template:



- 1 Select **Load Template** from the lower navigation bar. To scroll through the list of saved templates use the navigation arrows  / . The corresponding template is displayed on the sample template panel.



Figure 4.21: Selecting a separation template. "Expt\_2a" was selected.

- 2 Select and highlight the desired template; in this case **Expt\_2a**.

#### 3 Select **Ok**.

**Note:** The sample separation template was loaded; however, the reagent rack has not yet been configured. It is necessary to configure the reagent rack before starting the run.



Figure 4.22: The template "Expt\_2a" was successfully loaded.



To delete a separation template:

- 1 Select **Load Template** from the lower navigation bar.
- 2 Scroll through the list of saved templates use the navigation arrows ▲ / ▼.
- 3 Select the template for deletion.



Figure 4.23: "Expt\_3" template was selected for deletion.

- 4 Select **Delete Template**.



## 4.5 Reagent menu

The reagent menu is used to program any reagent vials that are required for automated magnetic labeling and subsequent cell sorting. Reagents can be entered using the 2D code reader or manually using the **Enter Reagent** input panel. For convenience it is possible to load and save reagent templates.



Figure 4.24: The reagent menu.

## 4.6 Log list menu



Figure 4.25: The “Log list” display.

The **Log list** records a complete log of actions performed by the autoMACS Pro Separator. An overview of the log list table follows:

Name	Definition	Name	Definition
Date	Indicates the date the action was performed.	Program	Name of program, for example: <b>Deplete:</b> Cell depletion <b>Possel:</b> Positive cell selection <b>Qrinse:</b> Quick rinse performed
Type	Description of the action, for example: <b>Rack:</b> Action involving rack <b>Init:</b> Initiation of instrument <b>Wash only:</b> Wash only performed <b>Service:</b> Service step performed <b>Special wash:</b> Special wash performed	Protocol	If a protocol is associated with the log, its name will be listed under this heading, for example: <b>CD4.h:</b> CD4 MicroBeads, human were used for autolabeling and cell separation.
Pos	Corresponding position on sample tube rack.	Status	The status of the log is depicted as follows: ✓ successfully completed ✗ action failed

To display further details about an individual log:

- 1 Select **Log list** tab and highlight a log from the log list.
- 2 Select **Details**. A detailed view of the program status is shown.
- 3 Select **Ok** to return to the log list screen or select **Log Details** to view a detailed log of performed actions.





Figure 4.26: Viewing log details.

A: A CD4 MicroBead positive cell selection was performed on March, 26th 2009.

B: An overview of program actions and corresponding times are displayed.

C: Details of each instrument action are listed.

## 4.7 Option menu



Figure 4.27: The "Option" display.

The **Option** menu allows maintenance procedures, such as exchange of autoMACS Columns or decontamination of the system. The menu is divided into two main categories, **Special** and **User settings**.

An overview of the functions available under each category are given below.

#### 4.7.1 Special

**Special** options comprise three special programs for column exchange (**Col\_ex**), instrument decontamination (**Safe**), and cleaning of the instrument for long-term storage (**Store**).

##### Safe

This is a disinfectant procedure which uses MACS Bleach Solution for cleaning and decontamination of the autoMACS Pro Separator. Depending on the level of use and general instrument maintenance, it is recommended to decontaminate the fluidic system every 3 to 6 months using the **Safe** program.

##### Store

The program **Store** should be applied to prepare the instrument for long-term storage. Upon completion of the **Store** program, the fluidic system contains 70% ethanol.

To store the autoMACS Pro Separator for a period longer than two weeks, the tubing system should be cleaned and the columns should be replaced with column substitutes. The **Store** program automatically performs the cleaning procedure and prompts the user to install column substitutes.

#### 4.7.2 User settings

The **User settings** are for maintenance and setup of the autoMACS Pro Separator. Each program is briefly discussed below.

##### About

**About** informs the user about the software version, serial number of the instrument, and other hardware information.

##### Calibr\_1: Performing “calibration 1” of the needle arm positioning

This program is used for the calibration of the needle arm to the washing station and tube racks. Refer to section 12.2 for more information.

##### Calibr\_2: Performing “calibration 2” of the instrument tubing

This program is used for the calibration of the instrument tubing. Refer to section 3.2.7 for more information.

##### Check\_up: Performing a system check-up

The **Check\_up** program allows the user to perform a system check-up. It is recommended to use the program if hardware errors occur. The program starts after highlighting **check\_up** and pressing **Run**.

## **O\_bcr: 2D code reader setup, configuration, and initialization**

The **O\_bcr** program allows the user to setup, configure, and initialize a recently installed autoMACS Pro Separator 2D code reader.

**Note:** In case of malfunction during cell separation this feature must be disabled in order to perform a cell rescue procedure.

## **O\_init: Optional priming of the instrument at startup**

By default, the autoMACS Pro Separator does not perform a wash program after initialization. The option **O\_init** allows the user to add an initial rinse program that will be performed automatically after each initialization to prime the instrument.

## **O\_led: Activating/deactivating bottle illumination**

The autoMACS Pro Separator has a bottle illumination designed to facilitate monitoring the instrument's status. The bottle illumination can be switched ON or OFF

## **O\_progs: Enabling/disabling special separation protocols**

The **O\_progs** are used to enable or disable special separation programs. To enable or disable these protocols, perform the following steps:

- 1 Select **Option**, **User settings**, and **O\_progs**.
- 2 Select **Run**. A dialog box will report the current status.

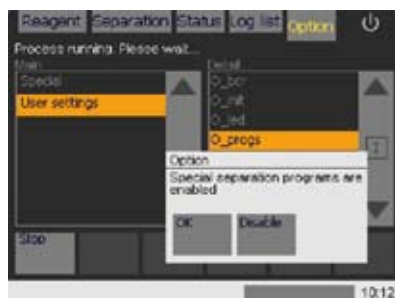


Figure 4.28: O\_progs: Enabling or disabling special separation protocols. Select "Disable" to disable special separation programs.

- 3 Select **OK** if the current reported status should not be changed. Alternatively, select **Disable** (or **Enable**) to change the status.

## **Sepcount: Displaying the number of performed column separations**

The **Sepcount** or **Sep. counter** program is used to display the number of column separations that have been on the autoMACS Pro Separator. To view this statistic, perform the follows steps:

- 1 Select **Option**, **User settings**, and **Sepcount**.
- 2 Select **Run**. A dialog box will report the number of separations.



Figure 4.29: Sepcount: viewing the number of performed separations. In this example, no separations have been performed using this instrument.

- 3 Select **OK** to return to the option menu.

### **Set\_time: Setting the time and date**

To set the time and date perform the following steps:

- 1 Select **Option**, **User settings**, and **Set\_time**. Select **Run**.
- 2 Highlight either the time or date fields by touching the display.
- 3 Use the keypad to change the date or time accordingly.
- 4 Select **OK** to return to the option menu. The procedure can be cancelled at anytime by selecting **Cancel**.

### **Valve\_ex: Exchanging the instrument values**

This program is used for valve exchange. Use of this program turns the valve to the exchange position for removal. Valves may require periodical exchange. Refer to section 7.4 for more details.



This chapter describes the consumables and accessories required for the operation of your autoMACS Pro Separator.

**IMPORTANT:** Please be advised that the autoMACS Pro Separator is specified for use with MACS MicroBeads, autoMACS Columns, and other genuine Miltenyi Biotec consumables and accessories only. Please only use consumables and accessories recommended by Miltenyi Biotec. Failure to use recommended consumables and accessories may result in inaccurate results, instrument malfunction or damage, premature wear, and reduced life time of the instrument. Miltenyi Biotec does not honor any warranty or accept any responsibility for damages resulting from the use of inappropriate consumables or accessories.

5.1 Materials required for operation

5.1.1 Solutions

For daily operation, the following solutions are required: Running Buffer, Washing Solution, and ethanol.

Solution	Description	Color code	Capacity	Order no.
Running Buffer	autoMACS Running Buffer or MACS Separation Buffer	Blue	6x1.5 L for 6x15 separations and rinsing cycles	130-091-221
Washing Solution	autoMACS Pro Washing Solution	Green	6x1.5 L for 6x15 rinsing cycles (Rinse, Sleep, Store, Safe)	130-092-987
Storage solution	70% v/v ethanol in distilled water (prepared from absolute ethanol)	Black		Not available

Table 5.1: Solutions required for daily operation.

Solution bottles can be identified by color code and symbols (table 3.1, Symbols and color-coding of fluid containers). For proper operation of the autoMACS Pro Separator, solution containers must be filled with a minimum volume of 150–200 mL. It is recommended to use ready-to-use MACS Buffers or fresh,



**Note:** MACS Running Buffer contains azide preservative. A solution without preservative may be prepared by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS Rinsing Solution (# 130-091-222)

**Note:** Do not use denatured ethanol (technical ethanol), as the autoMACS Columns are not resistant to oxidative compounds. Use 100% ethanol, analytical reagent grade, without additive.

filter-sterilized solutions to prevent potential contamination of the tubing system.

The autoMACS Pro Washing Solution is a filter-sterilized and ready-to-use solution to rinse the fluidic system after any autoMACS Pro Cell Separation. It contains a detergent that dissolves cell aggregates. The autoMACS Pro Washing Solution was developed for optimal cleaning of the autoMACS Pro Tubing System.

70% ethanol: This solvent should be prepared by diluting absolute ethanol with distilled water.

### 5.1.2 Hardware

#### MACS MiniSampler

The MACS Mini Sampler can be equipped with three different tube racks (for details, refer to table below). To attach the MiniSampler and the tube racks, refer to section 3. The autoMACS Pro Separator will automatically detect the MiniSampler and the type of tube rack.

#### Tube racks, tubes and reagent racks

Three tube racks, Chill 5, Chill 15, and Chill 50, designed for 5 mL, 15 mL, and 50 mL tubes, respectively, and are available for use with the autoMACS Pro Separator. All three racks contain a coolant. To cool the racks, keep them in a refrigerator for 3–4 hours. Do not cool below 0 °C to avoid freezing of the samples. Use 5 mL, 15 mL, or 50 mL tubes. The process has been optimized using BD Falcon™ tubes.

Rack type	Tubes	Maximum number of samples	Maximum sample volume
Chill 5	5 mL	6	2.5 mL
Chill 15	15 mL	5	12.5 mL
Chill 50	50 mL	3	Up to 50 mL
Reagent Rack 4	4 vials	Not applicable	Not applicable

Table 5.2: Cooling tube and reagent racks.

## 5.2 Materials required for maintenance

### 5.2.1 Solutions

#### MACS Bleach Solution

MACS Bleach solution (# 130-093-663) is used in combination with the Safe program. Depending on the level of use, it is recommended to run a Safe program at least every 3 to 6 months. If material like whole blood or tissue is primarily used it is recommended to run program Safe once a month.

#### Disinfectant solution

It is recommended to clean the ports of the automated arm and the

**Note:** Program Safe can also be used for decontaminating the autoMACS Pro fluidic system. For special decontamination procedures contact the technical support team for further advice.

surface of the instrument of spilled fluid with 70% ethanol or isopropyl alcohol on damp tissue. Alternatively, use alcohol swabs.

**Distilled water**

It is recommended to use distilled water on tissue to remove any salt crusts from the instrument.

**5.2.2 Hardware****autoMACS Columns**

Order no. 130-021-101 (5×2 columns)

Capacity of  $2 \times 10^8$  magnetically labeled cells from up to  $4 \times 10^9$  total cells.

**Column substitutes**

Order no. 130-090-835 (2 pieces)

For installation prior to storage of the autoMACS Pro Separator for longer than two weeks. The instrument is delivered with installed column substitutes.

**Hydrophobic 0.2 µm air filters**

Order no. 130-090-385

Hydrophobic air filters are used to vent fluid containers and to prevent aerosols. Do not use hydrophilic filters, since they are easily blocked upon contact with liquid and thus may cause errors.

**autoMACS Pump Syringe**

Order no. 130-090-339

(1–2 required per year)

**Wrench**

Order no. 130-090-378

For tightening tube connections. Included in the autoMACS Pro Starting Kit.

**autoMACS Pro Laminar Hood Plate**

Order no. 130-093-246

The metal plate has been developed for stable placement of the autoMACS Pro Separator on potentially bending surfaces; for example, the surface in a laminar flow hood or safety cabinet. The thick metal plate is designed to stabilize the instrument and maintain optimal alignment with the MACS MiniSampler.

**autoMACS Pro Angle Connector Set**

Order no. 130-093-245

The autoMACS Pro Angle Connector Set has been designed for placement of the autoMACS Pro Separator in a location with limited space. Cables that connect the back of the instrument, i.e., the fluid sensor cable, the MACS MiniSampler cable, and the 2D code reader cable are connected at a 90° angle to reduce the total depth of the instrument. The set contains three Sub-D-pin adaptors.



# Cell separation using the autoMACS® Pro Separator

The following chapter describes how to perform cell separations using the autoMACS® Pro Separator.

**IMPORTANT:** The autoMACS Pro Separator is intended for research applications only and not for diagnostic or therapeutic use.

The procedure of cell separation has been categorized into eight steps.

- 1 Select a cell labeling strategy:** Choose if autolabeling, manual labeling, or a combination of both strategies should be needed.
- 2 Prepare cell samples:** A single-cell suspension devoid of dead cells, aggregates, and cell debris is the prerequisite for efficient cell separation.
- 3 Select a cell separation strategy and a suitable magnetic labeling strategy:** Prior to cell separation samples are labeled by magnetic MicroBeads. This can be performed manually or automatically using the autoMACS Pro Separator. Cell separation strategies are classified as:
  - positive selection or depletion,
  - direct or indirect magnetic labeling,
  - cell sorting based on one or more marker(s).
- 4 Determine number of samples:** Select an appropriate tube rack for the number of samples and also take the sample volume into account.
- 5 Prime the autoMACS Pro Separator:** Before performing a cell separation, the autoMACS Columns must be filled with Running Buffer by running the Rinse program.
- 6 Define autoMACS Pro Separation Programs on the sample rack template:** Select a separation and a washing program for every cell sample or, labeling separation wash program

## Separation programs

- Positive selection or depletion
- Standard mode or sensitive mode
- Single or double column selection

- Special programs for efficient depletion of unwanted cells or cells that are only dimly labeled
- Special programs for cell separation directly from whole blood
- Special programs for the sequential depletion of up to  $14 \times 10^8$  cells

#### Washing programs

- **Quick Rinse (Qrinse)** or **Rinse** are washing steps between separations
- **Sleep**—to follow the last program before the instrument is switched off

**7 Run the autoMACS Pro Cell Separation:** The autoMACS Pro Separator automatically isolates target cells from up to six independent samples in a sensor-controlled manner.

**8 Shutdown the autoMACS Pro Separator:** A sleep or store program is used for overnight storage or long-term storage, respectively. Sleep or store must be performed before shutting down the autoMACS Pro Separator.

## 6.1 Select a cell labeling strategy

The autoMACS Pro Separator is capable of performing autolabeling, manual labeling, and a combination of both strategies. Up to six samples can be processed at one time. Autolabeling protocols are being continually developed and optimized by Miltenyi Biotec. For a current list of MACS Reagents and Kits that are optimized for cell separations with the autoMACS Pro Separator autolabeling feature, please visit: [www.automacspro.com](http://www.automacspro.com).

## 6.2 Prepare cell samples

In order to obtain optimal separation results, some crucial points must be considered:

**Note:** Use Pre-Separation Filters (# 130-041-407) when cell clumping is suspected, especially when working with previously frozen material, cord blood, dissociated tissue, or whole blood. Cell pre-filtration removes aggregates, dead cells, and debris.

### 6.2.1 Prepare single-cell suspensions

Cell aggregates may contain mixtures of target and non-target cells and therefore can impair the separation results. To minimize the risk of cell aggregation MACS Separation Buffer/MACS Running Buffer should be used during sample handling steps. Resuspend cells carefully after centrifugation. For specific recommendations, please refer to the general protocol section of the user manual or to the respective MACS Cell Separation Reagent data sheet.

Large cell aggregates may interfere with the separation process and may cause pressure variations in the autoMACS Pro fluidic system. It is recommended to use Pre-Separation Filters (# 130-041-407) to remove cell clumps that may clog the column.

Dead cells and cell debris may bind non-specifically to MACS MicroBeads, antibodies, and antibody conjugates. To remove dead cells, it is recommended to use density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

For specific recommendations, please refer to the general protocols in the corresponding section of this user manual or to the respective MACS Cell Separation Reagent data sheet.

### 6.2.2 Reagent volumes and labeling volumes must be adjusted to the total cell number

Typically,  $10^7$  cells are labeled in a total volume of 100  $\mu\text{L}$ . When working with higher cell numbers, scale-up all reagent volumes and total volumes accordingly. For example, for  $2 \times 10^7$  total cells use twice the volume of all indicated reagent volumes and total volumes indicated in the respective data sheet. When working with fewer than  $10^7$  cells, do NOT scale down the volume, use the same volume as indicated. For specific recommendations, please refer to the respective MACS Cell Separation Reagent data sheet.

For details concerning sample volumes required for the first autolabeling step refer to table 6.1 below.

MACS Product	Strategy	Reagents	Cell concentration	Minimal volume*	Minimal absolute cell number
Direct MicroBeads - human - rat - non-human primate	Positive selection or depletion	1	$10^7$ cells per 80 $\mu\text{L}$	160 $\mu\text{L}$	$2.0 \times 10^7$
Direct MicroBeads - mouse	Positive selection or depletion	1	$10^7$ cells per 90 $\mu\text{L}$	180 $\mu\text{L}$	$2.0 \times 10^7$
Whole Blood MicroBeads	Whole blood or bone marrow	1	Original volume	0.25 mL – 8 mL	
Cell Isolation Kits	Untouched selection	2	$10^7$ cells per 40 $\mu\text{L}$	160 $\mu\text{L}$	$4.0 \times 10^7$
Cell Isolation Kits	Untouched selection	3	$10^7$ cells per 30 $\mu\text{L}$	120 $\mu\text{L}$	$4.0 \times 10^7$
MicroBead Kits	Positive selection or depletion	2	$10^7$ cells per 60 $\mu\text{L}$	120 $\mu\text{L}$	$2.0 \times 10^7$

\* When working with fewer cells than the necessary minimal volume, resuspend cells in the stipulated minimal volume

Table 6.1: Table shows dilution volumes required for the first autolabeling step.

## 6.3 Select a cell separation strategy and a suitable magnetic labeling strategy

The autoMACS Pro Separator can perform fully automated sample processing, i.e., sample autolabeling, labeled cell incubation, cell separation, and target cell elution. The software also offers the choice to perform cell separation on manually labeled cells. Thus, it is possible to perform automated cell separation with autolabeling, automated cell separation without autolabeling, or automated cell separation using a combination of both strategies.

### 6.3.1 Choosing a cell separation strategy

There are two basic strategies for separating specific cell populations: positive selection and depletion. Using the positive selection strategy, the target cells are magnetically labeled and collected as the positive fraction. Using the depletion strategy, the unwanted cells are labeled, separated from the target cells, and eluted as a positive cell fraction, i.e., cells labeled with MicroBeads. The target cells will be collected in the negative fraction. Both strategies can be combined for multiparameter sorting.

#### Positive selection

Positive selection allows up to 10,000-fold enrichment of the magnetically labeled target cells. Positive selection takes advantage of the high specificity of monoclonal antibodies to isolate highly pure cell populations that specifically express the corresponding antigen. The positively selected cells are virtually unaffected by the separation procedure and can be used immediately for culturing or other applications. There is no need to remove the MACS MicroBeads from the cells. The small size and the composition of the MACS MicroBeads (iron oxide and polysaccharide) make them biodegradable. Typically, MACS MicroBeads do not activate cells or influence function or viability.

Positive selection is recommended:

- for highly specific labeling
- for excellent purity and recovery
- for the isolation of rare cells
- if no subsequent separation step is needed.

#### Depletion

For some experiments it may be desirable to deplete certain cell types from the cell sample in order to isolate the target cells. The depletion strategy allows, for example, to isolate a target cell for which no specific antibody is available or to isolate “untouched” cells. With a depletion strategy all unwanted cells are magnetically labeled. During magnetic separation, the labeled cells are retained on the column, while the target cells pass through and form the negative, non-labeled fraction.

A depletion strategy is recommended:

- for the removal of unwanted (non-target) cells
- if no specific antibody is available for the target cells
- if binding of minimal amounts of antibody to surface molecules can interfere with downstream applications
- if isolated cells are to be sorted subsequently utilizing a second marker.

### **Multiparameter sorting : depletion followed by positive selection**

Cells can also be isolated by first depleting the non-target cells, followed by magnetic labeling and positive selection of the target cells. This strategy is useful if unwanted cells in the cell suspension express the same antigen that was chosen as the marker for positive selection.

It can also be used to deplete non-target cells from the initial cell suspension before isolating extremely rare cell types by positive selection.

### **MACS MultiSort strategy**

With MACS MultiSort Kits, high numbers of cells characterized by multiple cell surface markers can be sorted easily and quickly. Even rare cells can be enriched efficiently.

Multiparameter sorting with MACS MultiSort Kits allows sequential positive selections of cells. The target cells are first labeled with MACS MultiSort MicroBeads and positively selected for the first parameter. Subsequently, the cells are incubated with the MultiSort Release Reagent, which enzymatically removes the MicroBeads from the antibodies. In the next step, these cells are magnetically labeled with MicroBeads conjugated with an antibody directed against a second marker. After labeling, the cells are again magnetically separated.

### **Magnetic labeling strategies**

There are two basic approaches to magnetic labeling: direct labeling with MACS MicroBeads and indirect labeling with MACS MicroBeads against primary antibodies, other specific ligands or their conjugates. Both approaches can also be used for a MACS MultiSort strategy.

### **Direct magnetic labeling**

Direct magnetic labeling is the fastest way of magnetic labeling. It requires only one labeling step as the specific antibody is directly coupled to the magnetic particle. Direct labeling minimizes the number of washing steps and, therefore, avoids cell loss. Highly specific monoclonal antibodies have been selected by Miltenyi Biotec to produce a large variety of antibody-conjugated MicroBeads targeting many human, mouse, rat, and non-human primate cell surface markers. The high specificity of the antibodies allows low background and easy optimization of the separation. Fluorescent staining using fluorochrome-conjugated antibodies can simultaneously be performed for subsequent analysis of the separated fractions by flow cytometry or fluorescence microscopy.



### Indirect magnetic labeling

Indirect labeling is recommended when no direct MicroBeads for a particular cell surface marker are available. Almost any monoclonal or polyclonal antibody or other specific ligand targeting any cell type from any species can be used for indirect labeling. Cells are first incubated with a primary antibody ligand that is unconjugated, biotinylated, or fluorochrome-conjugated. In a second step, magnetic labeling is performed by using Anti-Immunoglobulin, Anti-Biotin, Streptavidin, or Anti-Fluorochrome MicroBeads, respectively.

A cocktail of antibodies or other ligands can also be used to concurrently isolate or deplete a number of cell types. As it results in an amplification of the magnetic label, indirect labeling may be the method of choice if weakly expressed markers are targeted for magnetic separation. When using a fluorochrome-conjugated antibody or other ligand in combination with the corresponding Anti-Fluorochrome MicroBeads, the fluorescent staining can be used for flow cytometric analysis.

### Cell isolation directly from whole blood or bone marrow samples

MACS Whole Blood MicroBeads are specially developed to isolate human cell subsets directly from human whole blood using the autoMACS Separator or the autoMACS Pro Separator. This reagent can also be applied to bone marrow. Note that in this case fat must be removed prior to labeling.

### 6.3.2 Choose a magnetic labeling strategy

Autolabeling may be performed prior to cell separation. For a list of MACS Products optimized for autolabeling please visit: [www.automacspro.com](http://www.automacspro.com)

**Note:** Titration and manual incubation with the primary antibody or other ligand is recommended to be done manually. If there is no wash step between incubation of the sample with an unknown primary antibody or other ligand followed by application of indirect MicroBeads, the procedure cannot be optimized. Results may be impaired.

### Installing reagents for use with autolabeling

The reagent menu is used to program any reagent vials that are required for automated magnetic labeling and subsequent cell sorting. Reagents can be entered using the 2D code reader or manually using the **Enter Reagent** input panel.



Figure 6.1: The reagent menu.

## Entering reagent information for autolabeling

Reagents vials can be directly scanned and recognized by the autoMACS Pro Separator 2D code reader.

### Scanning reagents with the 2D code reader

- 1 Select **Reagent** tab and highlight the position where the vial will be placed on the reagent rack. Four positions are available: **R1**, **R2**, **R3**, and **R4**.



Figure 6.2: Position “R1” was selected for the reagent vial.

- 2 Activate the reader by selecting **Read Reagent** and present a reagent vial in front of the 2D code reader. Ensure the 2D code is facing the blinking code reader–light. The optimal reading distance is 0.5–2.5 cm from the code reader–cover, tilt the vial as depicted in figure 6.3.



Figure 6.3: Scanning a reagent vial using the 2D code reader. In this example, the vial “CD4 Microbeads, human” was scanned.

- 3 The vial is automatically recognized by the software. The next reagent rack position will be automatically highlighted (**R2**).

**Note:** If the reagent vial cannot be identified by the 2D code reader please enter the reagent information manually; refer to “Entering reagents manually” below.



Figure 6.4: CD4 MicroBeads, human (# 130-045-101) was identified by the 2D code reader software and assigned to the reagent rack position “R1”.

- 4 Using the same procedure another reagent vial can be scanned using the 2D code reader. The reagent rack position R3 is automatically assigned. Having entered the desired reagent(s), click the **Separation** tab to proceed with programming a cell separation.

### Entering reagents manually

- 1 Select **Reagent** tab and highlight the position where the vial will be placed on the reagent rack. Four positions are available: **R1**, **R2**, **R3**, and **R4**.

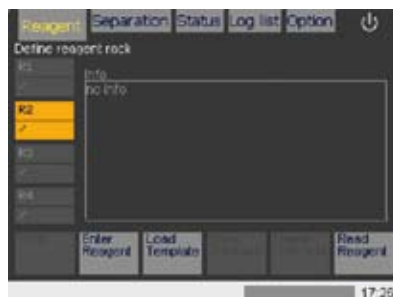


Figure 6.5: Position “R2” was selected for the reagent vial.

- 2 Select **Enter Reagent** from the lower navigation bar. Enter the reagent-specific product order number. The order number is located on the product data sheet. In the event that the data sheet is misplaced, visit [www.miltenyibiotec.com](http://www.miltenyibiotec.com) to download a printable PDF of the document.



Figure 6.6: Reagent information is manually entered using the reagent order number.

- 3 Select **OK**.
- 4 If a correct number is inserted the software will immediately recognize the reagent or kit. To confirm, select the reagent from the list by using the touch screen.

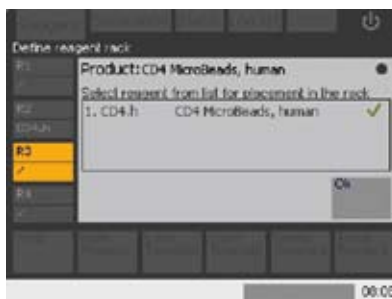


Figure 6.7: CD4 MicroBeads, human (# 130-045-101) was manually entered.

- 5 Select **OK** to confirm the identified reagent and reagent vial position "R2".



Figure 6.8: Reagent vial “CD4 MicroBeads, human” was assigned to the reagent rack position “R2”.

### Deleting reagents or reagent lists

In this example the following reagents were scanned into the following reagent rack positions:

- R1: FcR Blocking Reagent, human (component 1/3 of product Monocyte Isolation Kit II, human; # 130-091-153)
- R2: Monocyte Antibody Cocktail , human (component 2/3 of product Monocyte Isolation Kit II, human; # 130-091-153)
- R3: Anti-Biotin MicroBeads (component 3/3 of product Monocyte Isolation Kit II, human; # 130-091-153)
- R4: CD4 MicroBeads, human; # 130-045-101

To delete an individual reagent from the reagent rack list:

- 1 Select the reagent name that must be deleted. In this example CD4 MicroBeads, human on position R4 must be removed.
- 2 Select **Delete Selected** to remove the highlighted reagent.



Figure 6.9: CD4 MicroBeads, human was assigned to reagent rack position R4, which was subsequently selected for deletion.

To delete the entire reagent list:

- 1 Select an unassigned position on the reagent rack. In this example position R4 was selected. (If there are no unassigned positions, delete an individual reagent as described above).



Figure 6.10: To delete all reagents from the reagent rack, an unassigned position on the reagent rack must be highlighted. In this example the only unassigned position "R4" was selected.

## 2 Select **Delete All**.

### Working with reagent templates

For convenience it is possible to load and save reagent templates. In this example the following reagents were scanned into the following reagent rack positions:

R1: CD4 MicroBeads, human (# 130-045-101)

R2: CD8a (Ly-2) MicroBeads, mouse (# 130-049-401)

R3: No reagent

R4: No reagent

To save a reagent template:

- 1 Assign reagent vials to reagent rack positions as outlined above (refer to section: Entering reagent information for autolabeling).



Figure 6.11: Using the 2D code reader, two reagent vials were assigned to the reagent rack positions "R1" and "R2".

## 2 Select **Save Template**.

- 3 Allocate a name to the template. In this example the template was saved as "EXPT\_2" (experiment 2).



Figure 6.12: Using the alphanumeric keypad assign a name to the template.

#### 4 Select **Ok**.

To load a reagent template:



- 1 Select **Load Template** from the lower navigation bar. To scroll through the list of saved templates use the navigation arrows  / . The corresponding template is displayed in the adjacent panel.



Figure 6.13: Selecting a reagent template. Expt\_2 was selected which comprises of CD4.h and CD8.m on positions R1 and R2, respectively.

- 2 Select and highlight the desired template; in this case "Expt\_2".

#### 3 Select **Ok**.



Figure 6.14: The template "Expt\_2" was successfully loaded.

To delete a reagent template:

- 1 Select **Load Template** from the lower navigation bar.

- 2 Scroll through the list of saved templates using the navigation arrows ▲ / ▼.
- 3 Select the template for deletion.
- 4 Select **Delete Template**.

### Cell separation at a glance

- 1 Assign MicroBead reagent vials to positions on the MACS Reagent Rack. It is recommended to use the 2D code reader. Alternatively, the product order number may be manually programmed.

**Note:** For a detailed introduction to the separation menu refer to section 4.4.



Figure 6.15: CD4 MicroBeads, human vial was placed onto the MACS Reagent Rack position "R1".

- 2 On the **Separation** tab the recommended separation strategy and wash program are pre-selected for the chosen autolabeling reagents.
- 3 Program the cell separation protocol using the **Separation** tab menu. For full details refer to section 4.4.

**Note:** "/" option under **Labeling** denotes that NO autolabeling will be performed. Only a cell separation will be performed; manual magnetic labeling must be performed prior to placing the sample on the Chill Rack.

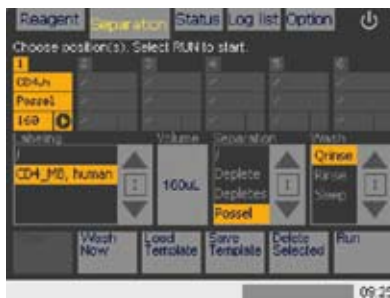






Figure 6.16: Performing MicroBead CD4<sup>+</sup> cell separation with autolabeling (left) and without autolabeling (right). The cell separation (Possel) and wash conditions (Qrinse) for both processes are identical. Disabling autolabeling ("/") influences the initial sample volume. For manual labeling it is recommended to dilute cells to a volume of 500  $\mu\text{L}/10^8$  total cells (refer to corresponding data sheet for further information).

**Note:** Refer to the respective product data sheet for more detailing on sample magnetic labeling volumes.

#### 4 Select Run.

##### Manual labeling at a glance

Cells are labeled with MACS MicroBeads according to the MACS Cell Separation Reagent data sheet. For general immunomagnetic cell separation protocols, please refer to section 8.1. The specific MACS Cell Separation Reagent data sheets can be found at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

### 6.3.3 autoMACS® Pro Cell Separation programs

The autoMACS® Pro Cell Separation programs are based on different cell separation strategies. There are ten pre-set separation programs to choose from depending on the cell isolation strategy, the frequency of target cells, and the level of antigen expression.

#### Positive selection programs:

**POSSEL**—Positive selection in standard mode: isolation of cells with normal antigen expression and frequencies higher than 5%; select Possel if purity is the highest priority.

**POSSEL\_S**—Positive selection in sensitive mode: isolation of cells with low antigen expression and frequencies higher than 5%; select Possel\_s if yield is the highest priority.

**POSSELD**—Positive selection in standard mode I, double-column program: for isolation of rare cells in low elution volume.

**POSSELDS**—Positive selection in sensitive mode, double-column program: for isolation of rare cells with low antigen expression.

**POSSELD2**—Positive selection in standard mode II, double-column program: for isolation of rare cells if purity is the highest priority.

**POSSELWB**—Special positive selection in special mode, double-column program: for isolation of cell subsets from whole blood. Cell samples are automatically diluted with Running Buffer.

**Depletion programs:**

**DELETE**—Depletion in standard mode: for removal of cells with normal to high antigen expression and results in better target cell yield.

**DEPLETES**—Depletion in sensitive mode I: removal of cells with low antigen expression and results in better target cell purity.

**DEPL05**—Depletion in sensitive mode II: removal of cells with low antigen expression and results in stringent depletion of cells.

**DEPL025**—Depletion in sensitive mode III: removal of cells with low antigen expression and results in stringent depletion of cells.

**A\_DEPL07**—Depletion in standard mode via loading of sample in separate 1 mL stages: removal of cells with normal to high antigen expression and results in a better target cell yield. This special program is disabled by default. To enable select **Option, User settings, and O\_progs.**

**A\_DEPLS7**—Depletion in sensitive mode via loading of sample in separate 1 mL stages: removal of cells with low antigen expression and results in a better target cell purity. This special program is disabled by default. To enable select **Option, User settings, and O\_progs.**

**Positive selection programs:**

In programs **Possel and Possels**, the magnetically labeled target cells are retained in the autoMACS Column 1. The unlabeled cells are released into the negative fraction collection tube (row “B” of the tube rack). After automated retraction of the magnet, the magnetically labeled cells are eluted into the positive fraction collection tube (row “C” of the tube rack).

In the double-positive selection programs **Posseld, Posselds, Posseld2, and Posselwb**, the magnetically labeled target cells are first retained in the autoMACS Column 1. The negative fraction containing the non-labeled cells is retrieved in the negative fraction collection tube (row “B” of the tube rack). Then, the magnetically labeled cells are held in a reservoir and loaded onto the autoMACS Column 2. Finally, the magnetically labeled cells are eluted into the positive fraction collection tube (row “C” of the tube rack).

**Depletion programs:**

When running any depletion program, the magnetically labeled non-target cells are retained in the autoMACS Column 1.

The non-labeled target cells pass through the column and are released into the negative fraction collection tube (row “B” of the tube rack). The magnetically labeled fraction, containing the unwanted cells, is eluted into the positive fraction collection tube (row “C” of the tube rack).

Program	Volume of non-labeled fraction (i.e. negative fraction)	Volume of labeled fraction (i.e. positive fraction)	Loading rate
Deplete	2 mL + sample volume	2 mL	4 mL/min
Depletes	2 mL + sample volume	2 mL	1 mL/min
Depl05	2 mL + sample volume	2 mL	0.5 mL/min
Depl025	2 mL + sample volume	2 mL	0.25 mL/min
A_Depl07*	3 mL per 1 mL sample volume	2 mL per 1 mL sample volume	4 mL/min
A_Depls7*	3 mL per 1 mL sample volume	2 mL per 1 mL sample volume	1 mL/min
Possel	2 mL + sample volume	2 mL	4 mL/min
Possel_s	2 mL + sample volume	2 mL	1 mL/min
Posseld	2 mL + sample volume	0.5 mL	4 mL/min (column 1) 1 mL/min (column 2)
Posseld2	2 mL + sample volume	2 mL	4 mL/min (columns 1 and 2)
Posselds	2 mL + sample volume	2 mL	1 mL/min (columns 1 and 2)
Posselwb	2 mL + sample volume + predilution volume	2 mL	4 mL/min (columns 1 and 2)

\*This program must be used with manual labeling only.

Table 6.2: Output volumes and loading rates of separation programs.

### 6.3.4 autoMACS® Pro Separator wash programs and maintenance programs

#### Wash programs for daily operation

The autoMACS® Pro Separator is equipped with reusable autoMACS Columns. After each cell separation, a thorough washing procedure rinses the columns of the autoMACS Pro Separator. After the wash program is completed, columns and tubing system are filled with Running Buffer.

Program	Washing Solution	Running Buffer	Storage solution	MACS Bleach Solution	Time
Qrinse	---	48 mL	---	---	1.5 min
Rinse	96 mL	48 mL	---	---	4 min
Sleep	96 mL	---	48 mL	---	5 min
Store	96 mL	---	96 mL	---	8 min*
Col_ex	96 mL	96 mL	---	---	6 min*
Safe	96 mL	96 mL	---	40 mL	21 min*§

\* Not including the time required for column exchange.

§ Not including the time required for disconnecting and reconnecting bottle tubing.

Table 6.3: Liquid usage and time of wash and maintenance programs.

**Qrinse** is the standard short wash program that only uses Running Buffer. It is recommended to use this program between separations of cells with normal frequency.

**Note:** The autoMACS Pro Separator will not start a separation program before a wash program has been completed.

**Rinse** is an extensive rinsing program that uses Washing Solution and Running Buffer. It is recommended to use this program between separations of rare cells, e.g., stem cells, the separation of cells from different species, and is mandatory between whole blood separations.

**Sleep**—it is mandatory to use Sleep as the last wash program before overnight storage. Upon completion of the Sleep program, the fluidic system contains 70% ethanol.

### Maintenance programs

The program **Safe** is designed for decontamination of the fluidic system. For details, refer to section 7. Upon completion of the **Safe** program, the fluidic system contains Running Buffer. The program includes an exchange of autoMACS columns.

The program **Store** should be applied to prepare the instrument for long-term storage. Upon completion of the **Store** program, the fluidic system contains 70% ethanol. For details, refer to section 7.

The program **Col\_ex** is used for column exchange. Upon completion of the **Col\_ex** program, the fluidic system contains Running Buffer.

## 6.4 Select appropriate tube rack

Three different tube racks are available for processing sample volumes between 0.2 mL and 50 mL. Unless otherwise specifically indicated in the MACS Cell Separation Reagent data sheet, the magnetically labeled cell samples are suspended at  $10^8$  total cells per 500  $\mu$ L when using manual labeling. The cell number and volumes for autolabeling are slightly different; refer to table 6.4 and table 6.5 below for further details.

- 1 Select the appropriate tube rack according to table 6.4 and table 6.5.
- 2 (Recommended) Cool down the tube rack for 3–4 hours in a refrigerator (2–8 °C) or until the coolant becomes solid. **Do not cool below 0 °C as samples may freeze.**
- 3 Equip the tube rack with sample tubes and fraction collection tubes.

**Note:** Row "A" of the tube rack holds sample tubes; row "B": tubes for non-labeled fractions; row "C": tubes for labeled fractions.




Rack type & symbol	Slots	Maximum number of samples	Minimum first incubation volume	Maximum final labeling volume	Maximum number of cells per tube*
Chill 5 	24x5 mL	6 (5 mL tubes)	0.2 mL	2.0 mL	2.0–4×10 <sup>8</sup> depends on cell labeling concentration and column capacity
			0.25 mL	1 mL	Whole blood only
Chill 15 	15x15 mL 5x5 mL	5 (15 mL tubes)	0.2 mL	6.5 mL	6.5×10 <sup>8</sup> –1.3×10 <sup>9</sup> depends on cell labeling concentration and column capacity
			1 mL	4 mL	Whole blood only
Chill 50 	6x50 mL 3x15 mL 3x5 mL	3 (50 mL tubes)	4 mL	8 mL	Whole blood only

Table 6.4: MACS Cooling Tube Racks specifications for autolabeling.




Rack type & symbol	Slots	Maximum number of samples	Maximum sample volume	Maximum number of total cells per tube
Chill 5 	24x5 mL	6 (5 mL tubes)	2.5 mL	5.0×10 <sup>8</sup>
Chill 15 	15x15 mL 5x5 mL	5 (15 mL tubes)	12.5 mL	2.5×10 <sup>9</sup>
Chill 50 	6x50 mL 3x15 mL 3x5 mL	3 (50 mL tubes)	50 mL	4.0×10 <sup>9</sup>

Table 6.5: MACS Cooling Tube Racks specifications for manual labeling.

## 6.5 Prime the autoMACS® Pro Separator

# 6

**Note:** It is assumed that the MACS MiniSampler has been already installed, the waste container is empty, and that all fluid containers are filled with recommended solutions (refer to section 3 for more details).



Figure 6.17: The initialization screen.

- 1 Switch on the autoMACS® Pro Separator and wait for the instrument to complete initialization.
- 2 After initialization, the autoMAC Pro Separator will display the menu **Status**. Verify the status of the instrument as outlined in section 4.3.
- 3 Ensure that the symbols for the fluid containers are colored green. If they are red, check whether the fluid containers are filled, the waste container is empty, and the color-coded bottle sensors are connected to the appropriate container. If symbols are gray, check the bottle sensors for proper connection.

**Note:** 70% ethanol does not contain electrolytes. Therefore, the fluid level cannot be determined and the symbol for this bottle is always gray. Please ensure that the volume of each solution is sufficient for the number of separation and washing programs to be performed.

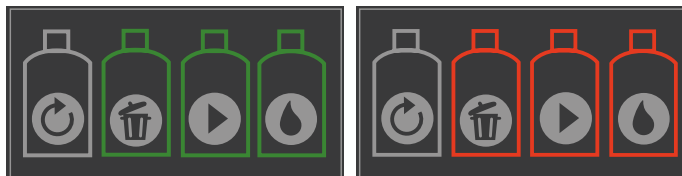


Figure 6.18: Fluid container status symbols.

Left: Fluid containers are ready. Right: Fluid containers need replaced.

- 4 Ensure that the symbol for the columns is colored green. For more details refer to section 4.3.2: Column status. If the symbol for the columns is red, the columns must be exchanged. The fill level on the symbol is an indicator for the remaining operation-life of the columns.



Figure 6.19: The column status graphic.

Left: The columns are ready. Right: The columns must be changed.

**Note:** The autoMACS Pro Separator automatically records the date of the last column exchange and displays the date for column replacement only if the program Column exchange has been used for column installation.

- 5 Ensure that the MACS MiniSampler is installed correctly. For more details concerning correct installation of the MACS MiniSampler refer to section 3.

**Note:** The touchscreen displays the type of tube rack upon starting the separation.



Figure 6.20: MACS MiniSampler status graphic.

Left: The MiniSampler was successfully installed. Right: No MiniSampler was detected.

- 6 The autoMACS Pro Separator is now ready for priming. Select **Separation** and **Wash Now** from the lower navigation bar.



Figure 6.21: "Wash Now" command displays two options: "Qrinse" (quick rinse) and "Rinse". A full rinse is required for instrument priming.

- 7 Select **Rinse** and **Run**.

## 6.6 Define autoMACS® Pro Separation program sequence or template

After completing the priming process or if a wash program has been completed, the autoMACS Pro Separator is ready for separation. The status of the instrument is displayed in the menu status. The bottles are illuminated green. Select the **Separation** menu.

- 1 Select the desired position(s) in the sample separation template field by touching it.
- 2 Select an autolabeling program and the sample volume, a separation program, and a washing program for each sample position. The selected programs will be displayed in the programming field.

**Note:** If priming of the instrument has not been performed, the autoMACS Pro Separator will automatically ask to run a **Rinse** program before starting the separation. The process can be monitored in the menu **Status**. At this point, the bottles are illuminated yellow.

It is possible to include an initial **Rinse** program that will be performed automatically upon switching on the instrument. The setting of this option is described in section 4.7. **O\_init:** Optional priming of the instrument at startup.

**Note:** Refer to sections 4.4 and 4.5 for an overview of the separation and reagent tabbed menus, respectively.

**3** (Optional) Templates can be saved by selecting **Save template** from the lower navigation bar. Follow the prompt to enter a template name.

**4** Select **Ok** and **Run**.

To select a particular sample position in the programming field, touch it once. To deselect it, touch it once again.

If the same combination of separation program and washing program is required for more than one sample, highlight all the desired sample positions first and then select the programs. Alternatively, define the program combination for one position and then highlight the other desired positions to adopt the program combination.

To erase selected program combinations from the programming field, first mark the respective positions and choose **Delete selected** from the lower navigation bar. If positions are not marked, the button can be used to delete the entire template. In this case, the option **Delete selected** will switch to **Delete all**.

Alternatively, start the separation from a previously saved separation template.

- 1** Select **Load template** from the lower navigation bar.
- 2** Select the desired template by using the arrows.
- 3** Select **OK**.
- 4** Select **Run** to start the separation. Check that there is enough buffer for the number of programmed separations.
- 5** Select **Continue**.

The autoMACS Pro Separator automatically detects the type of tube rack in use and allows the user to utilize only the number of samples and sample positions the tube rack can handle. If the tube rack does not match the template definition, a warning screen will be displayed upon starting the separation.

### 6.6.1 Entering sample separation instructions: a walkthrough example

In the following example two samples were placed on positions 1 and 2 of a Chill Rack 15. Sample 1 was already manually labeled using CD4 MicroBeads, human. Sample 2 has not yet been labeled; the autoMACS Pro Separator will perform autolabeling using CD4 MicroBeads, human.

- 1** Highlight sample positions 1 and 2 on the sample template.

**Note:** For autolabeling the Reagent Rack configuration must be programmed using the **Reagent** tab menu. Refer to section 4.5 or 6.3.2 for further details.

To obtain brief information on the separation and wash programs, highlight the program of interest and press the information button located between the arrows. A pop-up window will be displayed. Alternatively, refer to section 6.3.3 for more information on separation programs.





Figure 6.22: Sample positions 1 and 2 were selected. The purpose of this example is to demonstrate how sample processing conditions can be easily modified and to clarify the differences between autolabeling and manual labeling. Alternatively, each sample could be programmed individually.

**Note:** The MACS Reagent CD4 MicroBeads, human was identified by the 2D code reader (barcode reader). The vial was placed on position 1 of the MACS Reagent Rack. Refer to section 4.5 for full details on using the **Reagent** menu.

**2** Select **CD4\_MB.human** under the submenu **Labeling**. By default the software selects a **Possel** (positive cell/target cell enrichment) separation strategy and a **Qrinse** (quick rinse) wash protocol.



Figure 6.23: The current display indicates that autolabeling will be performed on both samples.

**3** Select the **Volume** submenu to enter the required sample volume. Select **Enter**.

For this experiment a minimal starting volume of 160  $\mu\text{L}$  is required for autolabeling (for CD4 autolabeling 40  $\mu\text{L}$  MicroBeads is added to the entire 160  $\mu\text{L}$  sample volume). Refer to the data sheet if this information is unknown.

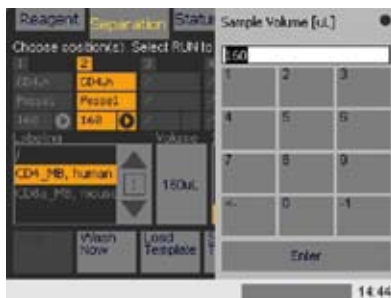



Figure 6.24: A volume of 160  $\mu\text{L}$  was entered for both sample positions.

- 4 Highlight the wash mode to be performed before cell separation. In this case a quick rinse (**Qrinse**; ) was selected.



- 5 Deselect sample position 2 by touching the display at this position.  
Sample position 1 must be setup to perform cell separation on a sample that was previously manually labeled with CD4 MicroBeads.

**Note:** Both samples positions have been setup for autolabeling.


**Note:** Sleep, , can only be selected as the last step in the program sequence.



Figure 6.25: Only sample position 1 is selected.

- 6 Deselect autolabeling for sample 1 by selecting "/" in the **Labeling** submenu. Change the sample volume as recommended in the data sheet. For manual labeling it is recommended to dilute cells to a volume of 500  $\mu$ l /  $10^8$  total cells (refer to corresponding data sheet for further information).



Figure 6.26: Only a positive cell separation will be performed on sample 1.

- 7 View and recheck the sample setup.
- 8 Select **Run** to start.
- 9 Check buffer levels as instructed by a popup dialog box. Click **Continue** to proceed.

10 It is recommended to monitor the process from the **Status** menu.  
Click the **Status** menu.



Figure 6.27: Sample processing is underway as instructed.

## 6.7 Monitoring the autoMACS® Pro Separator during cell separation

The autoMACS® Pro Separator is a sensor-controlled instrument that allows easy monitoring during operation.

### 6.7.1 Status menu before separation

Refer to section 4.3 for an overview of how to monitor the instrument status using the **Status** menu prior to performing a cell separation. Nevertheless, a brief summary is included below.

**Note:** Refer to section 4.3 for more information for clarification of the **Status** menu.

#### Status of fluid containers

The status of fluid containers is indicated by color-coded graphic symbols and by a text table.

Container	Symbol	Symbol color and user action
Running Buffer		Green: no action required Red: refill container Gray: connect bottle sensor
Washing Solution		Green: no action required Red: refill container Gray: connect bottle sensor
Storage solution		Gray: no liquid detection; visually check volume
Waste		Green: no action required Red: empty waste Gray: connect bottle sensor

Table 6.6: Status of fluid containers displayed in the “Status” menu.

### Column status

If the column symbol is green, no action is required. If the symbol is red, the columns must be exchanged. The level of the green fill on the column symbol indicates the remaining service-life of the autoMACS Columns.

### Rack detection

Rack detection only occurs upon starting the separation process.

### MACS MiniSampler detection

If the MACS MiniSampler has been detected correctly a rectangular symbol is displayed. If it has not been detected a hand symbol will be displayed in the same field.

## 6.7.2 Status menu during cell separation

It is recommended to monitor the instrument's status during cell separation using the **Status** menu. Programs yet to be processed appear in lilac. Programs currently undergoing autolabeling appear in lilac. Programs in progress in which no autolabeling is being performed switch to orange; completed programs switch to white. The current action is always displayed in the status bar located below the lower navigation bar. The status bar also displays the overall progress in minutes (min).



Figure 6.28: Monitoring the status during cell separation. Sample processing at position 1 is completed (white color). Sample at position 2 has finished the separation and is now performing a Rinse.

### Interrupting cell separation

The cell separation process can be paused at any time from all menu screens by selecting **Stop**.

- 1 From any menu screen, select **Stop**. The autoMACS Pro Separator will then immediately stop operation and will display a pop-up warning screen from which the current program can be continued or canceled.



Figure 6.29: Select “Stop” to pause cell separation. To continue cell separation select “Continue”. To cancel the entire procedure, select “Cancel”.

- 2 Select **Cancel** to cancel the procedure. Alternatively, select **Continue** to carry on with cell separation.



Figure 6.30: The process has been cancelled.

### Bottle illumination

The autoMACS Pro Separator has a bottle illumination that facilitates monitoring of the instrument’s status. The table below summarizes the color code of the bottle illumination and the respective user action required.

**Note:** Interrupting the process after labeling will prolong the incubation period.

Code	Status	User action
Green	Ready for separation	No action required
Blue	Instrument operating	No action required
Yellow	Not ready for separation	Run wash program (Rinse or Qrinse) before starting a separation
Red	Error	Check screen for error detection
Purple	Program Sleep is completed	Switch off autoMACS Pro Separator
Blinking	Action required	Check screen for required action

Table 6.7: Various bottle illumination statuses.

The bottle illumination can be switched ON/OFF.

- 1 Select the **Option** menu and **User settings**.
- 2 Highlight **O\_led** and press **Run**. The bottle illumination can now be enabled or disabled.

## 6.8 Shut down the autoMACS® Pro Separator

### 6.8.1 Sleep as the final wash program

- 1 Combine a separation program and the program **Sleep** for the last position in the programming field.
- 2 Upon completion of the **Sleep** program, switch off the autoMACS® Pro Separator using the main power switch.

**Note:** If **Sleep** is chosen as a wash program, the autoMACS Pro Separator will not allow definition of any programs beyond this position.

The autoMACS Pro Separator automatically performs a **Sleep** program if the device is inactive for more than six hours.

Ensure that enough Storage Solution is in the bottle.

### 6.8.2 Store: the program for long-term storage

To store the autoMACS Pro Separator for a period longer than two weeks, the tubing system should be cleaned and the columns should be replaced with column substitutes. The **Store** program automatically performs the cleaning procedure and prompts the user to install column substitutes.

- 1 Select **Option** and **Special**.
- 2 Select **Store** and press **Run**. The system will be rinsed automatically.



Figure 6.31: Running the "Store" program.

- 3 Install the column substitutes (refer to section 3.3.4 for details on column exchange).
- 4 Select **Done**.
- 5 Switch off the autoMACS Pro Separator using the main power switch.

### 6.8.3 Shutdown button

- 1 Press the shutdown button on the upper right hand corner of the screen (⏻).

2 Select **Yes** from the popup dialog box.



Figure 6.32: Shutting down the autoMACS Pro Separator.

- 3 The autoMACS Pro Separator will automatically perform a **Sleep** program.
- 4 Upon completion of the **Sleep** program, you will be prompted to shut down the instrument. The bottle illumination is purple at this point. Switch off the autoMACS Pro Separator by using the main power switch. **Note:** Ensure enough storage solution is available.

Appropriate maintenance of the autoMACS Pro Separator helps to maintain excellent reproducibility of the cell separation results. The following section gives you an overview on the procedures required for efficient maintenance of the instrument.

**IMPORTANT:** Insufficient or improper maintenance of your autoMACS Pro Separator can cause unpredictable results, avoidable malfunction and premature failure of the instrument, and may void your warranty.

**IMPORTANT:** Please do not perform any maintenance procedures other than specifically described in this user manual. Any other maintenance procedures must be performed by qualified service personnel.

**Inquire with your local Miltenyi Biotec representative about Miltenyi Biotec's extensive instrument service and support arrangements, or refer to [www.miltenyibiotec.com/support](http://www.miltenyibiotec.com/support).**

**IMPORTANT:** When replacement or spare parts are required for maintenance, only use genuine Miltenyi Biotec parts or third-party parts specified and recommended by Miltenyi Biotec. Using unauthorized replacement or spare parts can cause malfunction of the instrument and impair cell separation results. Miltenyi Biotec does not honor any warranty or accept any responsibility for instrument failure or damages resulting from the use of inappropriate replacement or spare parts.

**CAUTION:** During a maintenance procedure, potentially contaminated liquid may spill out of the orifice of the washing station and the tubing. Therefore, wear protective gloves, protective clothing, and safety glasses to avoid contact with skin and eyes. Dispose used gloves and clothing appropriately.

## 7.1 General considerations

Please consider that the sample quality has a significant influence on the system's performance. It is crucial to use single-cell suspensions for cell separation. Furthermore, dead cells and other small particles potentially derived from the sample preparation should be removed prior to cell separation. For further information on sample preparation, refer to section 6.1.



**Note:** Do not use a dishwasher to clean and do not autoclave any of the removable parts unless indicated otherwise.

## 7.2 Daily maintenance

The following maintenance procedures must be performed on a daily basis. If the instrument is not to be used for an extended period of time the store procedure must be performed; refer to 7.2.3 for further details.

### 7.2.1 Instrument priming

A Rinse program must be performed before performing the first cell separation after the instrument has been switched on.

Performing an instrument rinse

- 1 Click the **Separation** menu and **Wash Now** from lower menu bar.
- 2 Select **Rinse** (💧).
- 3 Click **Run**.

#### **O\_init: Optional priming of the instrument at startup**

By using the O\_init program, the instrument can be instructed to perform an automated rinse sequence at startup. The Rinse program is used for efficient washing and equilibration of the fluidic system.

- 1 Select **Option, User settings**, and **O\_init**. Press **Run**.
- 2 Follow the prompt on the screen to enable or disable the initial wash.

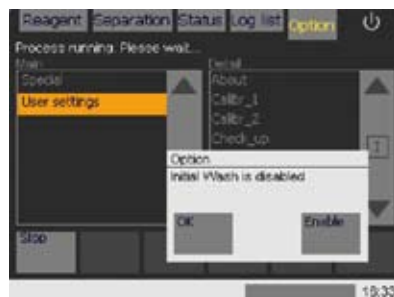


Figure 7.1 Initial wash program is used to activate or deactivate an automatic instrument prime on instrument startup.

**Note:** In case of malfunction during cell separation this feature must be disabled in order to perform a cell rescue procedure.

The instrument automatically prompts the user to perform a rinse before performing a cell separation.

### 7.2.2 Clean uptake/outlet ports

It is recommended to clean uptake and outlets daily. This should be performed before running the **Sleep** program (shutdown).

- 1 Switch the instrument OFF.
- 2 The ports can be wiped with tissue soaked with 70% ethanol, isopropyl alcohol, or MACS Bleach Solution followed by distilled or deionized water.

- 3 Remove the finger-guard that is attached underneath the outlet port holder by pulling gently. Clean the finger-guard as described for the ports.

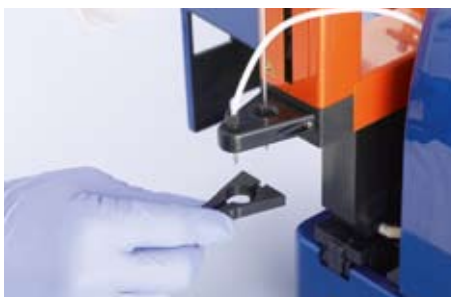


Figure 7.2: Removing the finger guard for cleaning.

- 4 Wipe the outlet port for the negative fraction as indicated above. The port can be flushed by using a syringe.
- 5 To clean the uptake port, ensure the instrument is switched OFF. Move the needle holder up and down to get access to the entire surface of the needle.
- 6 Push the finger-guard back into position.
- 7 Switch the instrument ON.
- 8 Run the Sleep program

**Note:** To prevent the formation of salt deposits, wipe the outlet ports with a tissue soaked with distilled (or deionized) water before each Sleep program.

### 7.2.3 Instrument shutdown

Make sure to run a Sleep program prior to switching the instrument OFF. This program ensures most efficient preservation of the fluidic system by rinsing with storage solution.

#### Shutdown button

- 1 Press the shutdown button on the upper right hand corner of the screen (⏻).
- 2 Select **Yes** from the popup dialog box.



Figure 7.3: Shutting down the autoMACS Pro Separator.

- 3 The autoMACS Pro Separator will automatically perform a Sleep program.
- 4 Upon completion of the Sleep program, you will be prompted to shut down the instrument. The bottle illumination is purple at this point. Switch OFF the autoMACS Pro Separator by using the main power switch.

**Note:** Ensure enough storage solution is available.

### Store: the program for long-term storage

To store the autoMACS Pro Separator for a period longer than two weeks, the tubing system should be cleaned and the columns should be replaced with column substitutes. The Store program automatically performs the cleaning procedure and prompts the user to install column substitutes.

- 1 Select **Option** and **Special**.
- 2 Select **Store** and press **Run**. The system will be rinsed automatically.

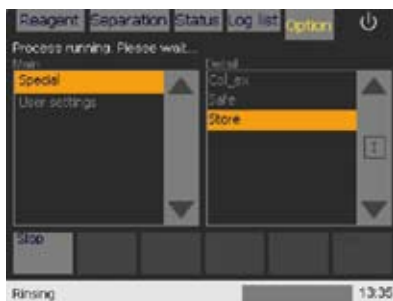


Figure 7.4: Running the "Store" program.

- 3 Install the column substitutes (refer to section 3.3.4 for details on column exchange).
- 4 Select **Done**.
- 5 Switch OFF the autoMACS Pro Separator using the main power switch.

## 7.3 Periodic maintenance

### 7.3.1 Column exchange

autoMACS Pro Separator Columns must be exchanged every two weeks or after 100 separations, whichever comes first. Refer to section 3.3.4 for more details.

### 7.3.2 Clean the pump syringe

Clean pump syringe must be cleaned every 1–3 months.

- 1 Run a **Sleep** Program (refer to section 7.2.3), switch OFF the power, and unplug the autoMACS Pro Separator from the electric supply. Open the front access cover. The syringe pump plunger should be at the top-right position.
- 2 Loosen the plunger lock screw by turning it counter-clockwise.



Figure 7.5: Unscrewing the plunger lock screw.

- 3 Lower the plunger holder by firmly pressing the plunger lock screw downward.

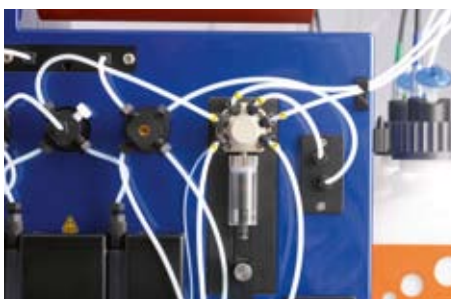


Figure 7.6: The plunger was gently lowered.

- 4 Unscrew the syringe from the dilutor valve housing.



Figure 7.7: To remove the pump syringe the top screw must be turned anti-clockwise.

- 5 Carefully remove the plunger from the syringe.
- 6 Remove salt crusts with distilled or deionized water.



Figure 7.8: Washing the pump syringe with distilled water.

**Note:** If symptoms of wear such as leakage persist, contact Technical Support.

- 7 Gently push the plunger back into the syringe and dry the plunger lock screw before proceeding with installation of the syringe.
- 8 To reinstall the syringe, carefully insert the syringe into the plunger holder.
- 9 Push the plunger holder up until the syringe reaches the dilutor valve.

- 10 Fasten the syringe at the dilutor valve by turning it until a resistance can be felt.
- 11 Push the plunger holder up once again and tighten the plunger lock screw.
- 12 Prime the autoMACS Pro Separator as described in section 7.2.1.
- 13 Run the program **Calibr\_2** to calibrate the fluidic volume control of the instrument. Refer to section 12.2 for details.

### 7.3.3 Clean the washing station

The washing station is designed for the automated rinsing of the outlet and uptake ports as well as surface cleaning of the uptake port needle. The washing station should be cleaned as necessary to remove spills and salt crusts.

- 1 Switch OFF and unplug the instrument.
- 2 Make sure that the needle arm is in the uppermost position.
- 3 Swivel the front cover to the right side; swivel the cover of the washing station to the left side. The cover can be removed by lifting it.



Figure 7.9: Removing the washing station.

- 4 Press the tubing clamp on the right hand side of the washing station and pull to remove the tubing.



Figure 7.10: Remove the peristaltic pump tube from the washing station.

**Note:** Potentially contaminated liquid may spill out of the orifice of the washing station and the tubing. Therefore, wear protective gloves, protective clothing, and safety glasses to avoid contact with skin and eyes.

- 5 Unscrew the thumb screw that attaches the washing station to the instrument.



Figure 7.11: Unscrew the thumb screw.

- 6 Pull out the washing station.
- 7 Clean the washing station by soaking it in 10% bleach and 70% ethanol for 15 min each. Optionally, sonicate it in water. Rinse with distilled water.
- 8 Reassemble the unit in reverse order.
- 9 Run a **Safe** program.

**Note:** Do not autoclave the washing station or wash using a dish washer.

**Note:** Run the Store program and exchange the autoMACS Columns with substitute columns if you intend to store the instrument for a period longer than two weeks (refer to section 6.8.2).

**Note:** If sample tubes and fraction collection tubes have been in contact with biohazardous material, they should be autoclaved after use.

Bottle closures can be autoclaved.

### 7.3.4 Instrument decontamination

The Safe program is a disinfectant procedure which uses MACS Bleach Solution for cleaning and decontamination of the autoMACS Pro Separator. Depending on the level of use and general instrument maintenance, it is recommended to decontaminate the fluidic system every 3 to 6 months using the Safe program. If predominantly whole blood, bone marrow, or tissue samples are separated the *Safe* procedure can be performed more often without harming the instrument, e.g., every four weeks instead of performing a column exchange.

Perform instrument decontamination according to the following instructions:

- 1 Select menu **Option**, **Special**, and the program **Safe**.

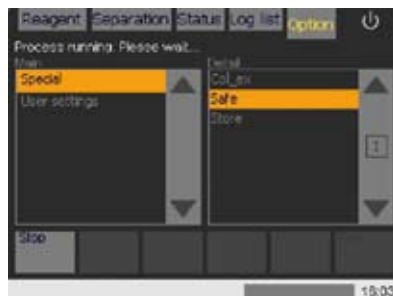


Figure 7.12: "Safe" program is underway.

## 7 Maintenance

**Note:** In case of spillage, it is recommended to use a disinfectant that is appropriate for the potential pathogen, e.g., bleach, isopropyl alcohol, or 70% ethanol, to decontaminate surfaces with tissue or swabs.

**Note:** Dispose tissues and swabs appropriately. It is recommended to wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Switch off and unplug the instrument beforehand.

- 2 Press **Run**. Follow the screen prompts.
- 3 Disconnect the tubings from all buffer bottles. Select **OK**.
- 4 Place the end of each tubings into a bottle of MACS Bleach Solution.
- 5 The decontamination procedure is performed automatically.
- 6 (Optional) Wash fluid containers and bottle closures using detergent, 1% hypochlorite, or 70% ethanol. Rinse thoroughly using deionized water.
- 7 Upon completion of the process, replace fluid containers, and reconnect all tubings.
- 8 Select **OK**. The system is rinsed automatically.
- 9 Wipe down the ports of the automated arm and the surface of the instrument using 70% alcohol swaps.

### 7.3.5 Instrument long-term storage

The program Store should be applied to prepare the instrument for long-term storage. Upon completion of the Store program, the fluidic system contains 70% ethanol.

To store the autoMACS Pro Separator for a period longer than two weeks, the tubing system should be cleaned and the columns should be replaced with column substitutes. The Store program automatically performs the cleaning procedure and prompts the user to install column substitutes.

- 1 Select the **Option**, **Special**, and **Store**. Select **Run**. The system will be rinsed automatically.

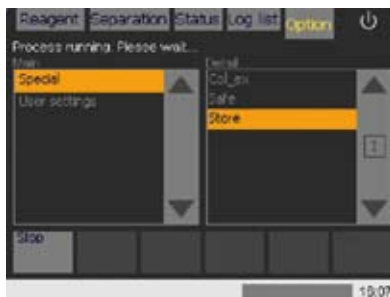


Figure 7.13: "Store" program is underway.

- 2 Install the column substitutes as described by the section 3.3.4.
- 3 Select **Done**.



- 4 Switch OFF the autoMACS Pro Separator using the main power switch.

## 7.4 Planned maintenance

### 7.4.1 Valve exchange

**Note:** It is recommended to exchange valves once a year.

The fluidic system of the autoMACS Pro Separator is regulated by six valves. The valve that is connected with the pump syringe is referred to as dilutor valve. If a valve of the autoMACS Pro Separator starts to leak, an exchange of the valve should be performed.

Exchange one valve at a time according to the following instructions:

**Note:** If wear of a valve is suspected, use the Check\_up program to analyze functionality.

- 1 Select **User settings** from the **Options** menu. Highlight **Valve\_ex** and press **Run** to start the program. Select valves which have to be exchanged. Valves will automatically be turned to exchange position.
- 2 Switch OFF and unplug the autoMACS Pro Separator. Open the front cover. For exchange of the lower valves, remove the bottom cover by pulling firmly.



Figure 7.14: The bottom cover must be removed for access to the lower valves.

- 3 Using the supplied wrench, unscrew the valve cover-screw which releases the tubing.



- 4 Detach all tubing and valve port locks or blind screws from the respective valve.



- 5 Loosen the two valve screws using the screwdriver from the autoMACS Pro Separator Starting Kit and pull out the valve.



- 6 Check if the groove in the valve drive is positioned horizontally and in the lower half of the axis.



Figure 7.15: Valve plate driving section.

1 = groove

2 = hole for adjustment pins

- 7 Make sure that the bracket of the new valve is positioned horizontally.

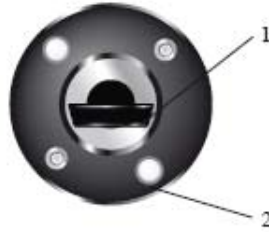


Figure 7.16: Rear view of the autoMACS Pro Separator valve.

1 = bracket

2 = adjustment pins

- 8 Carefully insert the new valve allowing the bracket to find the groove in the drive. At first, the bracket will slide in only halfway.



Figure 7.17: Inserting the new valve.

- 9 Gently rotate the valve. The two adjustment pins will slide into their corresponding holes in the valve plate.
- 10 Make sure that the valve is fully inserted into the driving station. Fasten valve screws using the screwdriver.
- 11 Connect the tubing with the installed new valve and fasten tubing by hand until finger tight.
- 12 Plug in and switch ON the autoMACS Pro Separator.
- 13 Perform a **Rinse** program and check the valves visually for leakage and air inlet.
- 14 Refasten tubing connectors using caution. If leakage persists call Technical Support.
- 15 Take care not to pinch the tubings at the bottom left of the instrument when closing the bottom cover.

**Note:** In case the valve exchange program **Valve\_ex** cannot be performed (e.g. valve does not turn any more) switch off instrument and turn the valve bracket manually to the position corresponding to the groove in the valve drive.

### 7.4.2 Exchange of dilutor valve

- 1 Switch OFF and unplug the instrument.
- 2 Remove the pump syringe as described in section 7.3.2.
- 3 Unscrew the tubing.

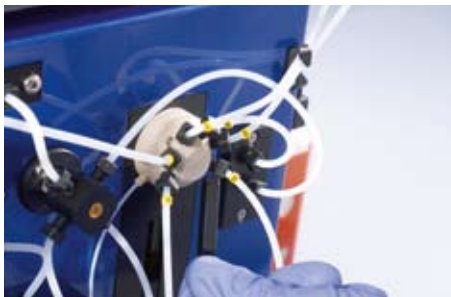


Figure 7.18: Unscrewing tubing connected to the dilutor valve.

**Note:** The profile of the valve shaft is shaped asymmetrically, similar to a trapezoid (refer to figure 7.19)

- 4 Unscrew the two hexagonal socket screws using the key that was delivered with the new or exchange valve. Take care to note the orientation of the valve shaft as shown below.



- 5 Pull the valve out of the coupling.
- 6 When inserting the new valve into the coupling, make sure that the short side of the trapezoid-shaped valve shaft points towards the triangle in the coupling.



Figure 7.19: Inserting a new valve: take care to note the orientation of the valve.

- 7 When the valve is properly inserted, mount the hexagonal socket screws.
- 8 Connect the tubing according to the positioning of the diluter valve.

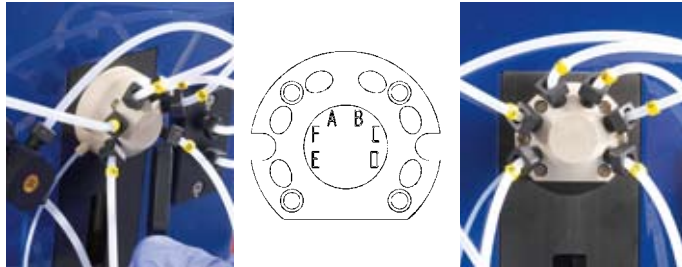


Figure 7.20: Connect tubing to the valve as shown above.

- 9 Guide the draining tube towards the washing station as shown below.

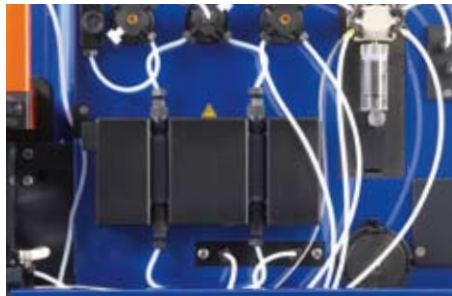


Figure 7.21: Red dashed line indicates the positioning of the draining tube. The draining tube connects the valve to the washing station.

- 10 Remove the washing station. Take care to clean spilled fluids with ethanol or disinfectant.





- 11 Carefully remove the waste distributor from its position by pulling in an upward direction and remove the draining tube from the old diluter valve.



Figure 7.22: The waste distributor.

- 12 Mount the new draining tube and finger tighten. Take care to clean spilled fluids with ethanol or disinfectant.



Figure 7.23: The draining tube is attached.

- 13 Install the waste distributor back to its former position and reinstall the washing station.
- 14 Install the syringe.
- 15 Switch ON the autoMACS Pro Separator.
- 16 Check for correct function by running the program **Rinse**.

- 17 Run the program **Calibr\_2** to calibrate the fluidic volume control of the instrument.

**Note:** The pump syringe should be exchanged once a year.

**Note:** The peristaltic pump head should be exchanged once a year.

#### 7.4.3 Exchange of the peristaltic pump head

- 1 Switch OFF and unplug the instrument.
- 2 Remove the washing station as described in section 7.3.3, steps 1–6.
- 3 Press the tubing clamp on the left hand side of the waste distributor and remove the tubing.



- 4 Pull out the bottom cover as depicted above.
- 5 Press clamps on both sides of the pump and pull out the pump head.



- 6 This will uncover a pin that protrudes from the instrument. Clean the pin using 70% ethanol but do not attempt to pull it out. The pin drives the pump during operation.
- 7 Replace pump head with the spare part.
- 8 Reassemble in reverse order. Take care not to pinch the tubing at the bottom left when pushing the bottom cover back into place.

#### 7.4.4 Exchange of hydrophobic air filter

Hydrophobic air filters (0.2  $\mu$ M) are attached to the bottle closures to vent the liquid bottles and to prevent release of aerosols. To avoid



clogging of the filters and to prevent contamination of liquids, air filters should be exchanged if they come into direct contact with any liquid, i.e., become wet. They also should be exchanged once a year to avoid clogging through dust deposits.

**Note:** The hydrophobic air filters should be exchanged once a year.

- 1 Hydrophobic air filters are attached to all fluid containers.



Figure 7.24: Hydrophobic air filter attached to a fluid container.

- 2 Unscrew the hydrophobic air filter from the fluid container. Replace the new filter in a similar manner.

#### 7.4.5 Exchange of autoMACS® Columns

The autoMACS® Columns should be exchanged after 100 separations or two weeks after the last column exchange, whichever comes first. The procedure is described in detail in section 3.3.4.

## 7.5 Exchange of the tubing system

If there is any leakage in the tubing system, the affected tubing part should be exchanged. Please note that each tubing has a specific length and should be exchanged exclusively with the corresponding spare part.

- 1 Switch OFF and unplug the instrument.
- 2 Remove the affected tubing by loosening the tube connectors using the black wrenches.
- 3 Replace the tubing with the correct part.
- 4 Pull back the connector from the tubing, so that the tubing can be easily inserted into the appropriate port.
- 5 Insert the connector cautiously and precisely and fasten it by hand. Make sure not to overwind the screw.



- 6 Plug in and switch ON the instrument. Run the program **Rinse** and check for leakages.
- 7 Run the program **Calibr\_2** to calibrate the volume control of the instrument. For details, refer to section 12.2.
- 8 If there are any leakages, refer to section 10.1.2 for advice on how to improve the tubing connection.

## 7.6 Exchange of fuses

If the instrument fails to start upon switching it on or if operation suddenly stops and the screen is dark, an exchange of the fuses might be required.

**CAUTION:** Replace fuses only with those of the same type and rating.

- 1 Switch OFF the instrument.
- 2 Unplug the main power cord from the power outlet as well as from the instrument. The fuse holder is located below the main power connector on the rear panel of the instrument.
- 3 Pull out the fuse holder from the housing and exchange fuses.



Figure 7.25: Removing the fuse holder.

- 4 Push the fuse holder back into the housing and reconnect the main power cord.

**Note:** Fuse specifications are given in section 11.2 and on the marking plate on the rear of the instrument close to the fuse holder. Do not use other fuses than specified.

## 7.7 Rescue procedure

Should the separation be interrupted before target cells are eluted, it is possible to perform a cell rescue procedure to recover the sample. If the instrument can be restarted, follow procedure A; if the instrument cannot be restarted, follow procedure B.

### 7.7.1 Rescue procedure A

- 1 Restart the instrument by switching it OFF and ON again.

- 2 Undo the tubing connector at the negative port and place into a 50 mL tube.
- 3 Take out the uptake port needle from the needle holder and place it into a 50 mL tube.
- 4 Undo the tubing connector of the waste tube at the waste bottle and place it into a 50 mL tube; place a second 50mL tube beside this one.
- 5 Run the program **Qrinse**. This will rinse the complete fluidic system with autoMACS Pro Running Buffer eluting the cells into the 50 mL tubes. Depending on which step of the separation program that the interruption occurred the cells will be found in any one of the vials.
- 6 Combine all fractions and centrifuge at 350×g for 10 minutes.
- 7 Discard the supernatant and apply cells to a reseparation as soon as possible. Keep cells on ice until the separation.
- 8 Reconnect all tubing at the appropriate positions and reposition up-take needle in needle holder

**Note:** Depending on the nature of your sample it is recommended to wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Dispose tissues, swabs and vials appropriately.

#### 7.7.2 Rescue procedure B

If it is not possible to restart the instrument the cells retained on the columns can be recovered.

- 1 Switch OFF the instrument and disconnect from the power supply.
- 2 Prepare two 50 mL tubes in rack and fill two 5 mL syringes with Running Buffer/Separation Buffer.
- 3 Open the front cover and place absorbent tissue underneath the columns.
- 4 Pull out the column from the column holder and replace the top connector with a 5 mL syringe filled with Running Buffer.
- 5 Undo the bottom connector and flush the column into a 50 mL tube. Discard column and syringe appropriately.
- 6 Repeat steps 4 to 5 with the second column.
- 7 Centrifuge tubes at 350×g for 10 minutes.
- 8 Discard the supernatant and perform reseparation on the recovered cells as soon as possible. Keep cells on ice until the separation.

- 9 Install new columns or column substitutes in place of the discarded ones.

## 7.8 Spare parts list

Part order number	Spare part	Part order number	Spare part
130-022-101	Pump Seal	130-093-307	autoMACS Pro Bottle Closure, red
130-090-684	4-port 4-way Valve	130-093-308	Dilutor Valve, 6-port distribution
130-090-685	4-port Distribution Valve	130-093-309	autoMACS Pro Tube (t32)
130-090-387	Air Filter Connector	130-093-310	autoMACS Pro Tube (t26)
130-093-291	autoMACS Pro Bubble Sensor	130-093-311	autoMACS Pro Tube (t20)
130-093-285	autoMACS Pro Tube (t2)	130-093-312	autoMACS Pro Tube (t12, t14)
130-093-287	autoMACS Pro Tube (t21)	130-093-313	autoMACS Pro Tube (t3)
130-093-284	autoMACS Pro Tube (t27)	130-093-314	autoMACS Pro Tube (t18)
130-093-288	autoMACS Pro Tube (t5)	130-093-315	autoMACS Pro Tube (t11, t25)
130-093-286	autoMACS Pro Tube (t7)	130-093-316	autoMACS Pro Tube (t6, t8, t23)
130-093-289	autoMACS Pro Tube, reservoir (t9, t24)	130-093-317	autoMACS Pro Tube (t10, t16, t17, t19, t22)
130-093-290	autoMACS Pro Uptake Port Needle	130-093-349	Washing Station, needle arm
130-090-676	Column Connector	130-093-350	autoMACS Pro Tube (t1, t13)
130-090-834	Fuse Holder	130-093-362	Tube Connector 2x2 Ports (1/4"-28 UNF)
130-090-385	Hydrophobic Air Filter	130-093-364	Tube Guiding Ball, needle arm
130-090-386	O-Ring for Bottle Closure	130-093-365	Peristaltic Pump Head incl. Tube
130-090-644	Plunger Lock Screw	130-093-366	Outlet Port Unit, needle arm
130-090-389	Power Cord (D)	130-093-367	Guard, needle arm
130-090-391	Power Cord (USA)	130-093-368	Thumb Screw (M5x16)
130-090-339	Pump Syringe	130-093-370	Magnet Lock, front cover

Part order number	Spare part	Part order number	Spare part
130-090-378	Square Key	130-093-371	4-port 3-way Valve
130-091-996	Valve Blind Screw, 5 pcs.	130-093-372	autoMACS Pro Tube (t31)
130-093-293	autoMACS Pro Tube (t4)	130-093-397	Uptake Port Needle Guiding, needle arm
130-093-302	Fuse 5x20 T4A, set	130-093-407	Thumb Screw (M5x40), MiniSampler
130-093-303	autoMACS Pro Sensor Cable	130-093-669	Cover, MiniSampler
130-093-304	autoMACS Pro Bottle Closure, green	130-094-682	Pump Syringe, Hamilton, 5mL, Cavo XP
130-093-305	autoMACS Pro Bottle Closure, blue	130-094-729	Dilutor Valve, 6-port distribution, V2
130-093-306	autoMACS Pro Bottle Closure, black		

Table 7.1: List of available spare parts.

## 7.9 Accessories list

Accessory order number	Accessory	Accessory order number	Accessory
130-091-339	Air Filter Extension Set	130-093-412	MACS Cooling Tube Rack, 3xChill 15
130-093-245	autoMACS Pro Angle Connector, set	130-093-416	MACS Cooling Tube Rack, 3xChill 5
130-094-573	autoMACS Pro Autolabeling Upgrade Kit	130-093-413	MACS Cooling Tube Rack, 3xChill 50
130-093-305	autoMACS Pro Bottle Closure, blue	130-092-952	MACS Cooling Tube Rack, Chill 15
130-093-304	autoMACS Pro Bottle Closure, green	130-092-951	MACS Cooling Tube Rack, Chill 5
130-093-246	autoMACS Pro Laminar Hood Plate	130-092-953	MACS Cooling Tube Rack, Chill 50
130-094-289	autoMACS Pro Laminar Hood Set	130-092-990	MACS Cooling Tube Rack, set
130-093-532	autoMACS Pro Protection Cover	130-093-292	MACS Fluid Container (1.5 L)
130-093-303	autoMACS Pro Sensor Cable	130-094-574	MACS Reagent Rack 4
130-093-669	Cover, MiniSampler		

Table 7.2: List of available accessories.

**Note:** Detailed protocols and product documentation are provided on the data sheet for the particular MACS Cell Separation Reagents.

Work fast, keep cells cold, and use pre-chilled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

Volumes for magnetic labeling procedures described below are for up to  $10^7$  total cells. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for  $2 \times 10^7$  total cells, use twice the volume of all indicated reagents and double the total volume). When working with fewer than  $10^7$  cells, do NOT scale down the volumes.

Keeping the samples on ice may result in poor cell labeling. Temperatures higher than  $8^\circ\text{C}$  and/or longer incubation times may lead to non-specific cell labeling. Fluorescent labeling can be performed either before or after the separation has been completed.

The following chapter describes protocols that may be used with the autoMACS Pro Separator using manual labeling. Please visit [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for an up-to-date list of manual labeling and autolabeling protocols using the autoMACS Pro Separator.

There are two basic approaches to magnetic labeling: labeling with direct MACS MicroBeads or indirect labeling with MicroBeads against primary antibodies or their conjugates. General instructions for magnetic labeling are described in the following sections.

## 8.1 Direct magnetic labeling

A variety of antibody-conjugated MicroBeads targeting human, non-human primate, mouse, and rat cell surface markers are available for direct magnetic labeling. The general protocol given below is based on a 1:5 dilution of MACS MicroBeads. Please note that direct magnetic labeling of mouse cells is typically performed in a 1:10 dilution of MACS MicroBeads. For details, refer to the specific MACS Cell Separation Reagent data sheet.

- 1 Prepare a single-cell suspension and determine the cell number.
- 2 Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
- 3 Resuspend cell pellet in  $80 \mu\text{L}$  of fresh buffer per  $10^7$  total cells.
- 4 Add  $20 \mu\text{L}$  of MACS MicroBeads per  $10^7$  total cells.
- 5 Mix well and incubate for 15 minutes in the refrigerator ( $2-8^\circ\text{C}$ ).
- 6 (Optional) Add staining antibodies, e.g.,  $10 \mu\text{L}$  of MACS Fluorochrome-conjugated Antibodies, and incubate according to the data sheet.
- 7 Wash cells by adding  $1-2 \text{ mL}$  of buffer per  $10^7$  cells and centrifuge at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
- 8 Resuspend up to  $10^8$  cells in  $500 \mu\text{L}$  of buffer.
- 9 Proceed to magnetic separation (refer to section 5.3.5).

## 8.2 Indirect magnetic labeling

**Note:** Primary antibodies should be titrated to determine the optimal staining dilution. After incubation with primary antibodies, wash the cells carefully. If unbound primary antibodies have not been completely removed, they may inhibit labeling of cells with indirect MACS MicroBeads.

Indirect magnetic labeling is the method of choice, if direct MACS Cell Separation products are not available. Almost any monoclonal or polyclonal primary antibody can be used: A variety of Anti-Fluorochrome MicroBeads is available for magnetic labeling of cells stained with primary antibodies conjugated to VioBlue®, fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), peridinin chlorophyll protein (PerCP), or tandem molecules like PE-Cy5 or APC-Cy7. Anti-Biotin MicroBeads and Streptavidin MicroBeads are recommended when using biotinylated primary antibodies. Streptavidin MicroBeads must be used with biotin-free buffer for labeling. This is not necessary when using Anti-Biotin MicroBeads, since they do not bind free biotin. Anti-Immunoglobulin MicroBeads against human, mouse, rat, or rabbit immunoglobulins can be used in combination with unconjugated antibodies.

- 1 Prepare a single-cell suspension and determine the cell number.
- 2 Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
- 3 Resuspend cell pellet and incubate with the primary antibody according to the manufacturer's recommendations. For MACS Fluorochrome-conjugated or Biotinylated Antibodies, resuspend up to  $10^7$  total cells in 100  $\mu$ L of buffer and add 10  $\mu$ L of the respective antibodies.
- 4 Mix well and incubate in the refrigerator for 5–10 minutes (2–8 °C) or according to the manufacturer's recommendations. If fluorochrome-conjugated antibodies are used, incubate in the dark.
- 5 Wash cells to remove unbound primary antibody by adding 1–2 mL of buffer per  $10^7$  cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
- 6 (Optional) Repeat washing step.
- 7 Resuspend cell pellet in 80  $\mu$ L of buffer per  $10^7$  total cells and add 20  $\mu$ L of indirect MicroBeads per  $10^7$  total cells.
- 8 Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
- 9 (Optional) When using unconjugated or biotinylated primary antibodies, cells can be stained with a fluorochrome-conjugated antibody at this point.
- 10 Wash cells by adding 1–2 mL of buffer per  $10^7$  cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

**Note:** When using Anti-FITC MicroBeads or Streptavidin MicroBeads, resuspend cell pellet in 90  $\mu$ L of buffer per  $10^7$  total cells and add 10  $\mu$ L of MicroBeads per  $10^7$  total cells.

**Note:** Keeping the samples on ice may result in poor cell labeling. Temperatures higher than 8 °C and/or longer incubation times may lead to non-specific cell labeling.

**Note:** For higher cell numbers, scale up buffer volume accordingly.

- 11 Resuspend up to  $10^8$  cells in 500  $\mu\text{L}$  of buffer.
- 12 Proceed to magnetic separation (refer to section 5.3.5).

### 8.3 Direct magnetic labeling of human cells using MACS® Whole Blood MicroBeads

MACS® Whole Blood MicroBeads have been developed for positive selection of many human cell types from anticoagulated whole blood samples or bone marrow samples. Perform magnetic labeling with MACS Whole Blood MicroBeads according to the following general instructions. Refer to the specific MACS Cell Separation Reagent data sheet for more details.

- 1 Prepare the sample as outlined by the data sheet.
- 2 Add 50  $\mu\text{L}$  MACS Whole Blood MicroBeads per 1 mL anticoagulated whole blood. When working with larger or smaller blood volumes, scale all reagent volumes and total volumes up or down, accordingly (e.g. for 500  $\mu\text{L}$  of blood use half the volume of Whole Blood MicroBeads, i.e., 25  $\mu\text{L}$ ).
- 3 Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
- 4 Wash cells by adding 2–5 mL of buffer per mL whole blood and centrifuge at  $445\times g$  at room temperature for 10 minutes and slow deceleration (no brake).
- 5 Aspirate supernatant carefully. Do not disturb the cell pellet. Leave a small residual volume of supernatant (approximately 1–2 mm in height) to avoid cell loss.
- 6 Resuspend the cell pellet, estimate the volume, and dilute with the same volume of buffer and proceed to section 5.3.5.
- 7 Fluorescent labeling should be performed after the separation process.





# Quality control of separations performed with the autoMACS® Pro Separator

To evaluate any MACS Cell Separation, the separated cells can be analyzed with regard to purity, recovery, and viability. Using MACS MicroBeads, the magnetically labeled cells can be simultaneously stained with fluorochrome-conjugated antibodies. Antibodies of the same specificity can be used in most cases. MACS Fluorochrome-conjugated Antibodies are standardized to evaluate MACS Cell Separations. The stained cells can subsequently be analyzed by flow cytometry, fluorescence microscopy, or other techniques.

## 9.1 Recovery of cells

In most cases, the number of isolated cells will be compared to the number of cells theoretically expected from the heterogeneous starting population. To calculate the target cell recovery, take an aliquot from the magnetically labeled fraction just before starting the cell separation.

The target cell recovery, e.g., positive cells in the magnetically labeled cell fraction can be calculated as follows:

**Note:** To count the cells of the original fraction, collect an aliquot of the cell sample after magnetic labeling directly before the magnetic separation to analyze whether cell losses are due to centrifugation steps OR to magnetic separation. Also take counting statistics into consideration. The standard deviation when counting cells is  $N \pm N^{1/2}$ . Therefore, cell counting might be associated with large statistical errors.

$$\text{Target cell recovery (\%)} = 100 \times \frac{\text{No. of cells in pos. fraction} \times \% \text{ positive cells in pos. fraction}}{\text{No. of cells in orig. sample} \times \% \text{ positive cells in orig. sample}}$$

The overall cell recovery can be calculated as follows:

$$\text{Overall cell recovery (\%)} = 100 \times \frac{\text{No. of cells in pos. fraction} + \text{No. of cells in neg. fraction}}{\text{No. of cells in orig. sample}}$$

## 9.2 Purity of isolated cell population

For most experiments that follow the cell separation, it is necessary to document the purity of the isolated cell subset. It is recommended to analyze the cells by flow cytometry. Alternatively, fluorescence microscopy can be used. Cells can also be analyzed by immunocytochemistry.

**Note:** Efficient staining of the cells with regard to signal intensity and specificity are the pre-requisites for an accurate analysis. Magnetic cell separation using the MACS Technology is a highly sensitive method and often results in extremely pure cell populations. Make sure that the method used for analysis is sensitive enough to accurately analyze the purity.

Purity of the positively selected cell fraction:

$$\text{Purity} = \% \text{ positive cells in positive (magnetically labeled) fraction}$$

Purity of the depleted cell fraction:

$$\text{Purity} = \% \text{ negative cells in negative (non-labeled) fraction}$$

## 9.3 Viability of the cells

Different dyes are available to discriminate between live and dead cells. The most common method to discriminate between live and dead cells is based on trypan blue staining and analysis by light microscopy. Trypan blue crosses the cell membrane of dead cells and stains the cells. Live cells are not stained.

Propidium iodide (5.0 µg/mL) is most often used for flow cytometry and fluorescence microscopy. It crosses the permeable cell membrane of dead cells, enters the nucleus, and interacts with DNA. Therefore, the nucleus of dead cells is fluorescently stained. Other fluorescent dyes, for example, DAPI [4',6-diamidino-2-phenylindole] can be used depending on the properties of the flow-cytometer, i.e., its excitation wavelength capabilities, particularly in the UV range.

When working with fixed cells, it is recommended to use the Fixation and Dead Cell Discrimination Kit (#130-091-163) for both the cell fixation as well as the discrimination of dead cells.

The viability can be calculated as follows:

$$\text{Viability (\%)} = 100 \times \frac{\text{No. of live cells}}{\text{No. of live cells (live \& dead)}}$$

**Note:** It is recommended to use the program **check\_up** if general hardware errors occur. Select the **Options** menu from the upper navigation bar. Then select **User settings**. Highlight **check\_up** and press **Run**.

The program automatically analyzes the functionality of moving hardware components. A report is displayed after the analysis of each single component.

The procedure can be canceled after each step or continued by pressing **OK**. Following hardware components are analyzed: dilutor valve, valves 1–5, peristaltic waste pump, magnet 1–2, needle arm (movement along the z-axis and the y-axis), and MACS MiniSampler. Furthermore, the calibration data is checked.

In the unlikely event of problems using the autoMACS Pro Separator the following section aims to address any relevant issues. If the outcome of a cell separation procedure is deemed unsatisfactory, this may either be due to incorrect function of the instrument or to inappropriate sample preparation. Both of these factors are discussed in this section. At the end of this section, a list of numerically encoded errors and warning messages are presented along with user actions for troubleshooting.

This section addresses problems that are not indicated by a warning or error screen, but might occur during the separation or rinsing programs. Identify the problem and refer to the appropriate section.

## 10.1 Hardware problems not indicated by a warning or error screen

### 10.1.1 Column leakage

- 1 If a freshly installed autoMACS Column shows signs of leakage, check if the column is installed properly. The column should be inserted precisely into the column connector and fastened to the point of resistance. If this is not the case, loosen the column connector, insert the column precisely, and tighten the connector again.
- 2 Run the **Qrinse** program: select the **Separation** menu from the upper navigation bar and **Wash now** from the lower navigation bar. Select **Qrinse** and press **Run**. Check if the leakage persists. If so, unscrew the column and check if the luer connectors of the columns are damaged. If this is the case, exchange the leaking column with a new autoMACS Column (refer to section 3.3.4).
- 3 Check if the column connector is fastened properly. If not, use second wrench to counter and tighten another quarter-turn.
- 4 If the problem persists, contact Technical Support.

### 10.1.2 Tubing leakage

- 1 Identify the location of the leaky tubing by running the **Rinse** program:
  - From the Separation menu select **Wash Now** (lower menu bar).
  - Highlight **Rinse** and click **Run**.
- 2 Check whether the tubing is tightened properly. If this is not the case, tighten the tube connector. The connector should be inserted precisely.
- 3 If the problem persists, loosen the tube connector and pull back the connector from the tubing.
- 4 Check the ends of the tubing for wear and fissures. If necessary, replace tubing with the appropriate spare part. Insert the tubing into the appropriate port. Then cautiously insert and fasten the tube connector.
- 5 Run the **Rinse** program and check if the leakage persists. If so, unscrew the tubing and check if the screw thread is damaged. If this is the case, order and install new tubing.
- 6 If the problem persists, contact Technical Support.

**Note:** Do not remove the connector from the tubing.

**Note:** Please note that each tubing has a specific length and should be exchanged with the corresponding spare part only.

### 10.1.3 Pump syringe leakage

Verify that the Running Buffer has equilibrated to room temperature before performing a washing or separation program. Cold buffer will make the plunger seal constrict more than usual and may lead to leakage. Salt crystals may also damage the pump syringe seal. To clean the pump syringe refer to section 7.3.2 and retry. If the problem persists follow the guidelines below.

- 1 Run the **Sleep** program.
  - Press the shutdown button on the upper right hand corner of the screen (⏻).
  - Click **Yes** to shutdown the instrument.
- 2 Switch OFF the instrument.
- 3 Wash the pump syringe as described in section 7.3.2.
- 4 Switch ON the instrument and run a **Qrinse** program to ensure that the problem is solved:
  - From the **Separation** menu select **Wash Now** (lower menu bar).
  - Highlight **Qrinse** and click **Run**.
- 5 If the leakage persists, order and install either a new pump syringe (# 130-090-339) or a new pump seal (# 130-022-101). For details on the installation, refer to section 7.3.2.

**Note:** Depending on the level of use, the pump syringe should be cleaned every 1–3 months.

Appropriate maintenance and long-term storage assures that no salt deposits accumulate in the pump syringe. Salt deposits may cause wear of the pump seal and thus may lead to leakage.

The pump syringe should not run dry at any time. This can damage the pump seal and thereby may lead to leakage of the pump syringe.

**Note:** To prevent the formation of salt deposits, wipe the outlet ports with a tissue soaked with distilled (or deionized) water before each **Sleep** program.

#### 10.1.4 Pump syringe is filled with air during operation

- 1 If there is any air inlet into the pump syringe during operation, the correct proceeding of a separation will be impaired.
- 2 Check all tubings that are connected to the fluid bottles. Make sure that all tubings are fastened properly. If a screw thread is damaged, order and install new tubing.
- 3 Check if the hydrophobic air filters connected to the bottles are clogged. Clogging may cause positive or negative pressure in the fluid bottles, which can lead to pressure problems in the fluidic system. If filters are clogged, replace them with new hydrophobic air filters (refer to section 7.4.5).
- 4 Check if the connections and pump syringe are leaky (refer to section 10.1.3).
- 5 Check if the uptake port needle is connected correctly and no air inlet is possible. If not, unscrew and check screw threads. If they are undamaged, reinsert precisely and fasten. Then use the wrench to turn an extra quarter-turn. Do not to overwind the screw.
- 6 If the problem persists, contact Technical Support.

#### 10.1.5 Washing station overflow

- 1 Verify that the washing station is not clogged with salt deposits. Take out the washing station and clean as indicated in section 7.3.3.
- 2 Reassemble washing station and run a **Rinse** program.
  - From the **Separation** menu select **Wash Now** (lower menu bar).
  - Highlight **Rinse** and click **Run**.
- 3 If problem persists follow the steps below.
- 4 Make sure that the peristaltic waste pump works properly. Run the **check\_up** program:
  - From the **Option** menu select **User settings**.
  - Highlight **Check\_up** and click **Run**.
- 5 If the **check\_up** program reports a problem with the waste pump, remove the pump head and clean the pin that drives the pump (for details, refer to section 7.4.4). Clean the washing station (refer to section 7.3.3). Reassemble the unit and check whether the problem persists.
- 6 If the problem persists, replace the pump head (refer to section 7.4.4).

### 10.1.6 Outlet port is clogged

- 1 If the outlet ports are clogged, e.g., due to salt deposits, the elution process might be affected.
- 2 Wipe the outlet port with a tissue soaked with 70% ethanol or double-distilled water.
- 3 Flush the outlet port manually using a syringe filled with 70% ethanol or double-distilled water.

### 10.1.7 MACS® MiniSampler does not move properly

- 1 Check whether the guiding of the MACS® MiniSampler is connected properly to the connector at the autoMACS Pro Separator labeled "External CAN".
- 2 Check whether the bolt below the rack detection protrudes from the instrument. If this is the case, push it in and turn it clockwise to lock the bayonet mount.
- 3 Check the cable connection between MiniSampler and autoMACS Pro Separator. Check for cable damages.
- 4 Check whether the MiniSampler can freely move to both sides and check for any resistance or collision.
- 5 If the problem persists, contact Technical Support.

### 10.1.8 Touchscreen remains dark

- 1 Switch OFF the instrument, wait 5 seconds, and switch ON again. If the autoMACS Pro Separator still does not initialize, go to step 2.
- 2 Check if the power cord is plugged in correctly and if the electric power is switched on.
- 3 Replace the fuses (refer to section 7.6). Spare fuses are included in the autoMACS Pro Separator Starting Kit.
- 4 If the problem persists, contact Technical Support.

**Note:** If initial wash (**Rinse** program) is enabled, press **Stop** as soon as the **Status** menu appears on the screen. A window opens displaying that the process is paused. Press **Cancel**.

### 10.1.9 Disruption of power supply during cell separation

In the unexpected event of a power supply failure during the cell separation procedure, follow the rescue procedure to recover the cell sample trapped in the autoMACS Pro Separator fluidic system.

- 1 Switch ON the autoMACS Pro Separator.
- 2 Disconnect the outlet tubing from the waste cap and put the end of the tubing in a 50 mL tube. The rescued cell sample will be rinsed out at the waste port.

- 3 Select the **Separation** menu and **Wash now**. Select **Qrinse** and press **Run**. The cells will be rinsed out with Running Buffer.
- 4 Reconnect waste closure and waste bottle.
- 5 Perform the **Rinse** program to prime the autoMACS Pro Separator.
- 6 The rescued cell sample can now be centrifuged and subsequently be reprocessed with the autoMACS Pro Separator.

## 10.2 Performance errors not indicated by a warning or error screen

### 10.2.1 Output volumes are not correct

- 1 Check for air inlet and leakages in the fluidic system by running the **Rinse** program.
  - From the **Separation** menu select **Wash Now** (lower menu bar).
  - Highlight **Rinse** and click **Run**.
- 2 If the tubing is leaking, go to section 10.1.2.
- 3 If the pump syringe is leaking, go to section 10.1.3.
- 4 If the column is leaking, go to section 10.1.1.
- 5 If one or more valves are leaking, go to section 7.4.1.
- 6 Check whether all tubing connections including column connections, bottle connections, and connections at the pump syringe are fastened. Loose connections may allow air to enter the system and therefore affect the performance.
- 7 If the problem persists, run the **Calibr\_2** program.
  - From the **Option** menu select **User settings**.
  - Highlight **Calibr\_2** and click **Run**.

If the problem persists contact Miltenyi Biotec technical support.

### 10.2.2 Low cell viability in final fraction

- 1 Low cell viability can result from problems during both the cell preparation and the autoMACS Pro Cell Separation. Hence, also check section 10.3.
- 2 Check the 70% ethanol. Make sure that analytical reagent grade ethanol (without additives) is used to prepare the solvent.



- 3 Check the Running Buffer. Make sure that the appropriate buffer has been used and that no contamination has occurred.
- 4 Check Washing Solution. Make sure that the appropriate solution has been used and no contamination has occurred.
- 5 Check the pump syringe for contamination. If contamination is obvious, refer to section 10.1.3 and change columns afterwards. Check for contamination of the tubing system and refer to the appropriate section.
- 6 Run the **Safe** program to decontaminate the fluidic system.
- 7 Check the column status. The autoMACS Columns should be exchanged after 100 separations or two weeks after the last column exchange, whichever comes first. The procedure is described in detail in section 3.3.4.
- 8 If the problem persists, contact Technical Support.

### 10.2.3 Low purity of isolated cell population

- 1 Low purities can be caused by problems during both the cell preparation and the autoMACS Pro Separation. Hence, also check section 10.3.
- 2 Low purities can also result from using an inappropriate separation program or labeling approach. Please also refer to section 6.
- 3 Check the exchange date of the autoMACS Columns. Using the autoMACS Columns for longer than two weeks or for more than 100 separations within two weeks may affect purity.
- 4 Check whether the autoMACS Pro Separator has been set to **Sleep/Store** correctly. Inappropriate storage or allowing the autoMACS Pro Separator fluidic system to run dry will affect the columns and therefore the purity.
- 5 Make sure that the appropriate Running Buffer has been used.
- 6 Perform the **Qrinse** program. If pumps are filled with air during the process, refer to section 10.1.4.
- 7 Perform a test run using PBS as a mock sample and check output volumes. For correct volumes refer to table 6.2 in section 6.3.3. If volumes are not correct, go to section 10.2.1.
- 8 Run the **Safe** program (refer to section 4.7).
- 9 If the problem persists, contact Technical Support.

**When utilizing the autolabeling feature**

- 1 Check whether the uptake needle is positioned in the middle of the sample tube during the mixing procedure. If not, mixing will be inadequate.
- 2 Check whether the reagent volume (contents of reagent vial) is sufficient for labeling.
- 3 Check that the recommended maximal sample processing time of 90 minutes was not exceeded. In the event of an elevated room temperature the cooling capacity of the Chill Racks may be compromised. Higher labeling temperatures could cause non-specific binding which may lead to lower purities when using positive selection kits.

**10.2.4 Low recovery of isolated cells**

- 1 Low recoveries and low purities may be due to similar problems. Go to section 10.2.3, steps 1–4.
- 2 Low recoveries can also be caused by partial column blockage. Run the **Safe** program and exchange the autoMACS Columns (refer to sections 4.7 and 3.3.4).
- 3 Perform a test run using PBS as a mock sample and check output volumes. For correct volumes refer to table 6.2 in section 6.3.3. If volumes are not correct, go to section 10.2.1
- 4 If the problem persists, contact Technical Support.

**Especially when using autolabeling**

- 1 Check whether the uptake needle is positioned in the middle of the sample tube during the mixing procedure. If not, mixing will be inadequate.
- 2 Check whether the reagent volume (contents of reagent vial) is sufficient for labeling.
- 3 Check that the recommended maximal sample processing time of 90 minutes was not exceeded. In the event of an elevated room temperature the cooling capacity of the Chill Racks may be compromised. Higher labeling temperatures could cause unspecific binding which may lead to lower purities when using positive selection kits.

**10.2.5 Sample not or only partly taken up**

- 1 If the sample has been taken up only partly, check if the sample contains clumps larger than 1 mm. Continue with the separation of this sample. As soon as the separation is finished, continue with step 2. Before processing any remaining samples, use a Pre-Separation Filter (# 130-041-407) to remove the clumps from the sample.

- 2 Remove the sample tube. Wipe the uptake port needle with a tissue soaked with 70% ethanol. Run the **Qrinse** program.
- 3 Check for cell clumps in the tubing system. If cell clumps are suspected, run the **Safe** program.
- 4 For separating cells that tend to aggregate (e.g. tissue cells), it may be helpful to dilute the sample 1:2.
- 5 Remove the sample tube and run the **Qrinse** program. Check tubing system and pump syringe for air inlet.
- 6 Make sure that all tubing are connected properly, especially the tubing connected to the columns and to the buffer bottles.
- 7 Verify that the uptake port needle is connected correctly and that no air inlet is possible. Tighten if necessary.
- 8 Make sure that both columns are installed correctly.
- 9 Clean pump syringe as described in section 10.1.3 to remove salt deposits. Salt crusts might allow air to enter the fluidic system.
- 10 Check whether the hydrophobic air filters connected to the bottles are clogged. If filters are clogged, replace them with new hydrophobic air filters.
- 11 Run the **Check\_up** program to identify hardware malfunctions.
- 12 If problem persists, call Technical Support.

### 10.2.6 Reagent vial runs dry

As the liquid levels in the reagent vials is not controlled by the system before uptake of reagent, it will not be detected if the reagent volume provided in the vial is sufficient for the current separation process or not. Ensure enough reagent is present in all vials for all programmed processes. As the residual volume in the vial is about 20 µL, please be sure to overfill the vial with reagent accordingly.

## 10.3 Magnetic labeling and separation

### 10.3.1 Positive selection

**Positive cells have not been retained on the column**

- 1 Magnetic labeling of the cells is insufficient because the MicroBead concentration was too low. This may be due to the fact that either too much buffer was added to the cells or too much buffer was left on the cell pellet after centrifugation before adding the MicroBeads. Furthermore, the total number of cells

may have been miscounted. Use a ratio of MicroBeads and cells as stated in the respective MACS Cell Separation Reagent data sheet.

- 2 Labeling of cells was ineffective due to too much debris and/or dead cells in the sample. Debris and dead cells will non-specifically bind to all other components present. Antibodies and MicroBeads will be captured non-specifically and not be able to label the cells in the positive fraction sufficiently any more.
- 3 Labeling of cells was ineffective due to an incubation temperature lower than recommended. It is recommended to incubate cells in the refrigerator (2–8 °C) for labeling.
- 4 The number of magnetically labeled cells exceeds the column capacity. Calculate the number of magnetically labeled cells, for example, by staining with fluorochrome-conjugated antibodies, and subsequent fluorescence analysis. The number should not exceed  $2 \times 10^8$  cells per sample. If necessary, split the sample.
- 5 Cells were not labeled with MicroBeads because the MicroBeads were degraded. Check the expiration date. Check for sterility of the MicroBeads, if the vial has been opened before.
- 6 Cells were labeled, e.g., with fluorochrome-conjugated antibodies, prior to magnetic labeling with direct MicroBeads. When antibodies recognizing the same epitope are used for fluorescent and magnetic labeling, fluorochrome-conjugated antibodies and MicroBeads might compete for the binding sites. This can result in insufficient magnetic labeling. It is recommended to perform magnetic labeling prior to staining with fluorochrome-conjugated antibodies. Alternatively, use indirect MicroBeads.
- 7 A non-optimal separation program was chosen, e.g., Possel instead of PosselS for dimly labeled cells.

#### **Especially when using autolabeling**

- 1 Check whether the uptake needle is positioned in the middle of the sample tube during the mixing procedure. If not, inadequate mixing will be performed.
- 2 Check whether the reagent volume (contents of reagent vial) is sufficient for labeling.
- 3 Verify whether the sample volume was programmed correctly. A reduced input volume will result in insufficient labeling of cells.

#### **Low purity of magnetically labeled cell fraction**

- 1 Check for cell aggregates. Negative cells may be retained when forming clusters with positive cells and, thus, contaminate the positive fraction. Use buffers devoid of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in order to reduce formation of cell aggregates.

- 2 Dead cells in the cell suspension may non-specifically bind to MicroBeads and will then be co-enriched in the positive fraction. Remove dead cells before separation by using the MACS Dead Cell Removal Kit (# 130-090-101) or by Ficoll-Paque™ density gradient.
- 3 The concentration of MicroBeads or the temperature used for magnetic labeling were too high. It is recommended to dilute the MicroBeads according to the data sheet. Cells should be incubated with MicroBeads in the refrigerator (2–8 °C).
- 4 The total number of cells may have been miscounted leading to an inappropriate ratio of MicroBeads and cells. Use a ratio of MicroBeads and cells as stated in the respective MACS Cell Separation Reagent data sheet.
- 5 Incubation time with MicroBeads was too long, leading to background labeling. Reduce the incubation time to the recommended values (refer to individual MACS Cell Separation Reagent data sheet). Typically, an incubation time of 15 minutes is required when incubating in the refrigerator (2–8 °C). For exceptions, refer to the respective data sheet.
- 6 When target cells are extremely rare (<5% of total cells), few non-labeled cells which are retained non-specifically may constitute a high portion compared to the target cells. Perform a second separation without re-labeling to remove non-specifically retained cells or choose a double column separation like PosselD, PosselD2, or PosselDS.

#### **Especially when using autolabelling**

- 1 Check that the recommended maximal sample processing time of 90 minutes was not exceeded. In the event of an elevated room temperature the cooling capacity of the Chill Racks may be compromised. Higher labeling temperatures could cause unspecific binding which may lead to lower purities when using positive selection kits.
- 2 Check whether the correct sample volume was programmed. Higher sample input volumes will result in an excess concentration of MicroBeads leading to non-specific labeling.

#### **Recovery of magnetically labeled cells is low**

Refer to section 10.3.1, Positive cells have not been retained on the column.

#### **Viability of magnetically labeled cells is low**

Dead cells have been co-enriched in the positive fraction due to non-specific binding. Remove dead cells before separation by using the MACS Dead Cell Removal Kit. Refer to section 10.2.2. This is prone to occur if the number of dead cells in the starting material exceeds the number of target cells.

**Overall recovery is low**

Cells are often lost during washing steps. Determine the number of cells immediately before magnetic separation to check whether the low recovery is due to this or due to problems during the cell separation.

**10.3.2 Depletion****Too many cells are retained on the column**

- 1 Magnetic labeling of the cells was non-specific. In order to block non-specific binding, use MACS FcR Blocking Reagent, human (# 130-059-901) or FcR Blocking Reagent, mouse (# 130-092-575) before labeling.
- 2 The concentration of MicroBeads and/or the temperature used for magnetic labeling were too high. It is recommended to dilute the MicroBeads according to the data sheet. Cells should be incubated with MicroBeads in the refrigerator (2–8 °C).
- 3 Incubation time with MicroBeads was too long, leading to background labeling. Reduce the time for incubation to the recommended values (refer to the respective MicroBeads data sheet). Typically, an incubation time of 15 minutes is required at 2–8 °C. For exceptions, refer to the respective MACS Cell Separation Reagent data sheets.
- 4 Dead cells in the cell suspension may bind non-specifically to MicroBeads and will then be co-enriched in the positive fraction. Remove dead cells before separation by using the MACS Dead Cell Removal Kit (# 130-090-101) or by Ficoll-Paque™ density gradient.
- 5 Incubation temperatures were higher than recommended, typically 2–8°C, leading to non-specific binding.
- 6 Inadequately stringent separation programs were chosen, e.g., DepleteS instead of Deplete. Please refer to the respective data sheet for the recommended program.

**Especially when using autolabelling**

- 1 Check that the recommended maximal sample processing time of 90 minutes was not exceeded. If the room temperature is too high the cooling capacity of the Chill Racks may be compromised. Higher labeling temperatures could cause unspecific binding which may lead to lower purities when using positive selection kits.

**Non-labeled fraction shows low purity**

- 1 Magnetic labeling was insufficient due to a low expression level of the surface marker. It is recommended to use an indirect magnetic labeling system (for example MACS Anti-Biotin MicroBeads) in order to increase magnetic labeling of the cells.

- 2 The number of magnetically labeled cells exceeds the column capacity. Calculate the number of expected magnetically labeled cells. The number of cells should not exceed  $2 \times 10^8$  per sample. If necessary, split the sample.
- 3 Labeling of cells was ineffective due to an incubation temperature lower than recommended. Incubate the cells in the refrigerator (2–8 °C) for labeling.
- 4 Labeling of cells was ineffective due to too much debris and or dead cells in the sample. Debris and dead cells will non-specifically bind to all other components present. Antibodies and MicroBeads will be captured non-specifically and not be able to label the cells in the positive fraction sufficiently any more.
- 5 Cells were not labeled with MicroBeads because the MicroBeads were degraded. Check the expiration date. Check for sterility of the MicroBeads, if the vial has been opened before.
- 6 Labeling of cells was performed for a shorter time than recommended. The typical incubation time is 15 min. Please refer to the respective data sheet for more details.

#### **Especially by using autolabeling**

- 1 Check whether the reagent vial has enough reagent to perform magnetic cell labeling.
- 2 Check whether the uptake needle is positioned in the middle of the sample tube during the mixing procedure. If not, limited mixing could lead to low purity.
- 3 Refer to the respective data sheet for the recommended separation program.

#### **Recovery of target cells is low**

Refer to section 10.3.1.

#### **Overall recovery is low**

Refer to section 10.3.1.

### **10.3.3 Indirect MACS® MicroBeads**

#### **Positive cells have not been retained on the column**

- 1 The unbound primary antibody was not completely removed and inhibits magnetic labeling with indirect MACS® MicroBeads. Wash cells carefully (optionally, wash twice) by adding 1–2 mL of buffer per  $10^7$  cells, i.e., 10–20 times the incubation volume) after incubation with primary antibody. Centrifuge and remove supernatant completely, preferably with a pipette or by vacuum.
- 2 The concentration of the primary antibody is too low. Therefore, magnetic labeling is not sufficient to retain the desired cells on

the column. To avoid this, the primary antibody should be titrated carefully. For tips and hints on titration, refer to the FAQs under Customer Support at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

- 3 The isotype of the primary antibody is not recognized by the Anti-Immunoglobulin MicroBeads. Make sure to use appropriate MicroBeads.
- 4 Check the expression of the antigen/epitope with flow cytometry.
- 5 Antibodies may have degraded. Check the antibody for its function. Storage of diluted reagents at 4 °C or –20 °C may lead to degradation
- 6 Cells were not labeled with MicroBeads because the MicroBeads were degraded. Check the expiration date. Check for sterility of the MicroBeads, if the vial has been opened before. Antibodies may have degraded. Check the antibody for its function. Storage of diluted reagents at 4 °C or –20 °C may lead to degradation.

#### **Positive fraction shows poor purity**

- 1 The concentration of the primary antibody was too high, leading to non-specific binding of unwanted cells. The primary antibody should be titrated carefully. For tips and hints on titration, refer to the FAQs under Customer Support [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- 2 The primary antibody shows non-specific binding. Add blocking reagents such as BSA or immunoglobulin before labeling the cells.
- 3 The incubation time during labeling was too long. Do not exceed the incubation times specified in the respective data sheet.
- 4 Check the primary antibody for its specificity. When antiserum is used, it is recommended to pre-adsorb the antiserum, e.g., on cells which do not express the antigen, or to purify it by affinity chromatography, ammonium sulfate precipitation, ion chromatography, etc. in order to avoid non-specific cross-reaction.

**Note:** Generally, MACS Fluorochrome-conjugated Antibodies are used in a 1:11 dilution (resuspend up to 10<sup>7</sup> cells in 100 µL of buffer and add 10 µL of MACS Fluorochrome-conjugated Antibodies). For details or exceptions, refer to the MACS Reagent data sheet. If other than MACS Antibodies are used, carefully titer staining reagents. For tips and hints on titration, refer to the FAQs under Customer Support at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

## **10.4 Fluorescent staining**

### **10.4.1 Cells are poorly stained**

- 1 Fluorochrome-conjugated antibodies may have deteriorated. Store MACS Fluorochrome-conjugated Antibodies at 2–8 °C.
- 2 Fluorochrome-conjugated antibodies and MACS MicroBeads might compete for epitopes. Check the efficiency of the fluorescent staining on cell samples that are not magnetically



labeled. Especially for weakly expressed antigens, it might be necessary to use a fluorochrome-conjugated antibody that is directed against an epitope different from that recognized by the MicroBeads.

- 3 For indirect staining of biotinylated antibodies with streptavidin-fluorochromes, please note that buffers supplemented with fetal bovine serum (FBS) or bovine serum albumin (BSA) may contain free biotin which can inhibit streptavidin binding to the biotinylated antibody. Alternatively, use MACS Anti-Biotin-VioBlue, -FITC, -PE, or -APC. MACS Anti-Biotin antibodies do not bind to free biotin.

#### 10.4.2 Cells are excessively stained

- 1 High background staining may occur when the concentration of the staining reagents is too high and the incubation time for the staining procedure is too long. The use of the pre-titrated MACS Fluorochrome-conjugated Antibodies is recommended to achieve optimal results.
- 2 Check the staining reagent for its specificity.

**Note:** To prevent contamination, run a Sleep program before turning off the instrument.

### 10.5 Contamination of tubing system

- 1 Decontaminate the instrument: refer to section 7.3.4 for details.
- 2 If the problem persists, call Technical Support.

### 10.6 Problems indicated by error or warning screens

If errors or warnings are displayed on the screen of the instrument, please refer to the following table. If the below measures do not clear the fault, call Miltenyi Biotec Technical Support. To assist in the troubleshooting process please have the instrument serial number and details of the error message at hand (i.e. error number, module number, file and error line). If this is not possible, please go to the instrument Log List and view the Log Details taking care to note the displayed parameters exactly. To discuss the table below please call Technical Support.

Error code	Cause	Possible remedies
-5	Hardware module is not initialized. After a module malfunction an initialization of the module is necessary. This might also be a subsequent error if another error has been displayed shortly before.	Restart instrument. Please call Technical Support if error is thrown again.
-27	Standard valve initialization failed. Valve may be blocked or worn and cannot be turned correctly or valve drive is damaged.	Switch off the instrument, wait for 5 seconds, and switch the instrument on again. If error persists touch the button DETAILS. Exchange displayed valve. If error is displayed again call Technical Support.
-28	Motor rotation detection failed. Valve may be blocked or worn and cannot be turned correctly. Otherwise valve or magnet drive malfunction.	Touch button DETAILS. Exchange displayed valve if applicable. If error is displayed again or magnet is displayed call Technical Support.
-29	Valve rotation hindered. Valve may be blocked or worn and cannot be turned correctly.	Switch off the instrument for 5 seconds and turn it on again. If error persists, switch off instrument, loosen air filters on buffer/waste bottles by a half turn, exchange columns for dummy columns, verify that Washing Station is free of clogs and salt deposits (eventually clean Washing Station), verify that uptake needle is not clogged. Switch on instrument, if error persists, touch DETAILS. Exchange displayed valve if applicable. Make sure to prepare samples as specified. If error is displayed again call Technical Support.
-257	Needle arm movement has been hindered.	If object blocked movement, remove object. Switch off instrument. Wait 5 seconds. Switch on instrument. Otherwise or if error is displayed again call Technical Support.
-263	Unable to read rack bar code correctly	Check bar code on rack or try different rack. Make sure ambient or direct sun light does not hit sensor. Clean sensor to remove salt or dust using a cloth soaked in 70% ethanol or double distilled water and wipe dry.

Error code	Cause	Possible remedies
-264	Unable to read rack bar code correctly	Check bar code on rack or try different rack. Make sure ambient or direct sun light does not hit sensor.
-769	Diluter plunger could not be initialized. Syringe not mounted correctly, syringe damaged, or diluter valve not positioned correctly.	Check if syringe is fastened correctly. Exchange if broken or damaged. If error is displayed again call Technical Support.
-775	Diluter is not initialized. After a diluter malfunction an initialization of the module is necessary. This might also be a subsequent error if another error has been displayed shortly before.	Restart instrument. Please call Technical Support if error is thrown again.
-777	Plunger movement blocked because of column clogging, blocked tubing set, or any other cross-section constriction	Restart instrument and try a Rinse to wash out clogged material. If error persists run Safe program. Otherwise exchange diluter valve or standard valves depending on where a constriction is suspected. Please call Technical Support if error is displayed again.
-6006	Air intake during sample uptake although needle did not yet hit bottom of tube. Leakage of air into system in front of bubble sensor, liquid level has been overestimated, or needle did not move to bottom (as fast as necessary).	Make sure foam on top of sample is not higher than 5 mm above liquid level. Verify that sample was filtered before separation and that uptake needle is not clogged. Check for leakage at point the where the needle and tubing meet, and where the tubing to bubble sensor meet. Verify that connectors are appropriately fastened.
-6009	Unexpected air in system during sample uptake.	Check for leakage or air bubbles in tubing from uptake-needle to valve 1. Verify that connectors are appropriately fastened. Calibrate needle arm. Check for buffer supply. Otherwise call Technical Support.
-6216	The reagent designated for the current labeling process is not assigned a position in the reagent rack.	Provide all necessary reagents in reagent rack und correct reagent rack definition.
-7001	Needle could not be retreated completely.	Try reinitialization by touching RETRY. Otherwise call Technical Support.

Error code	Cause	Possible remedies
-7002	Collision of the needle with the bottom of the tube (or any other object) has been detected, but resetting the collision sensor failed although the needle has been lifted.	Push back needle to it's proper position in the needle holder, check for smooth running of the needle in its support then touch CONTINUE. Otherwise calibrate needle arm, especially if using a Chill 15 Rack.
-7003	Collision of the needle with an unexpected object. Resetting the collision sensor failed as the needle could not be lifted (already too close to top).	Check for objects hindering the movement of the needle. Remove the uptake needle from the needle holder and verify that there are no physical obstructions. Press CONTINUE.
-7004	Collision of the needle with an unexpected object far above expected tube bottom.	Check that cover of the washing station is properly closed. Remove any objects hindering the movement of the needle. If needle hits rim of tube or top of rack calibrate needle arm using Calibr_1 program. Touch LIFT for needle retreat.
-7005	Collision of the needle with an unexpected object far above expected tube bottom.	Check that cover of the washing station is properly closed. Remove any objects hindering the movement of the needle. If needle hits rim of tube or top of rack calibrate needle arm. Touch RETRY to try again.
-7006	Collision of the needle with an unexpected object far above expected tube bottom.	Check that cover of the washing station is properly closed. Remove any objects hindering the movement of the needle. If needle hits rim of tube or top of rack calibrate needle arm. Touch RETRY to try again.
-7007	Sample volume exceeds maximum volume specified for rack or program type. Remaining portion of sample will not be processed.	Do not use sample volumes exceeding the maximum volume specified for rack or program type. If volumes are in specified range but error is displayed anyway, please call Technical Support.
-7008	The sample volume has been underestimated. The needle has been rinsed but might still be contaminated.	Please clean outside of needle manually. If this error is thrown frequently please call Technical Support.
-7009	Collision of needle with bottom of tube could not be detected at expected position.	Check if tubes are correctly positioned in rack corresponding to template programming. Check MACS MiniSampler connection in front of autoMACS Pro Separator.

Error code	Cause	Possible remedies
-7010	Not certain if liquid surface of sample has been detected correctly.	Touch IGNORE to continue without liquid detection. Needle will be moved to bottom directly. This might result in a subsequent warning -7008 if the liquid level is higher than 60 mm above the tube bottom (see above). TOUCH retry to continue with liquid detection. Ensure that the tubing from the needle arm to bubble sensor can move freely. Adjust the tubing if necessary. Otherwise call Technical Support.
-7011	Restart of the device is required.	Please restart instrument.
-7012	Calibration data not found.	Please calibrate needle arm axes. Run program Calibr_1.
-7013	Calibration data not found.	Please calibrate needle arm axes. Run program Calibr_1.
-7014	Calibration data not found.	Please calibrate tubing. Run program Calibr_2.
-7015	Columns are not installed.	Please install columns.
-7018	Calibration data not found.	Please calibrate MACS MiniSampler using program Calibr_1.
-7021	A separation program has been started but system had not been rinsed properly.	Please rinse system by touching WASH or abort with CANCEL.
-7022	Columns are overdue.	Please install new columns. Touch CANCEL to abort and then install columns, touch CONTINUE to ignore and use old columns (not recommended).
-7023	Plunger movement blocked because of column clogging, blocked tubing set or any other cross-section constriction during output of the negative fraction. The negative fraction has not been eluted completely. Negative cells are still remaining in the system.	Touch CONT to discard the remaining negative cells into the waste bottle. To output negative fraction again at lower speed, exchange negative tube with an empty tube and touch RETRY.
-7024	The number of programmed sample positions exceeds the actual positions of the rack on the MACS MiniSampler.	Exchange rack with rack holding more samples and touch OK or touch CANCEL to abort and reprogram.
-7026	The protective cover of the MACS MiniSampler seems to be opened by the moving needle arm hitting the cover.	Check configuration and connection of the protective cover, the MACS MiniSampler, and the front support at the autoMACS Pro Separator then touch CONTINUE. Touch CANCEL to abort.

Error code	Cause	Possible remedies
-7027	The protective cover of the MACS MiniSampler needs to be closed.	Please close protective cover and touch CONTINUE. Touch CANCEL to abort.
-7028	Bar code on chill rack could not be read. MACS MiniSampler is not connected (properly).	Check electrical connection of MACS MiniSampler. If detected the MACS MiniSampler symbol would be displayed in the status screen.
-7029	Unable to read rack bar code correctly	Check bar code on rack or try different rack. Make sure ambient or direct sun light does not hit sensor. Touch RETRY to try again, touch SELECT to set chill rack type without automatic bare code reading.
-7030	The instrument has been shut down without using SLEEP.	Always use SLEEP to shut down the instrument.
-7031	The given whole blood sample size exceeds the maximum volume specified. The sample cannot be diluted sufficiently.	Do not use sample volumes exceeding the maximum whole blood sample volume specified for the used chill rack type. Split sample into several tubes, reprogram separation parameters and restart separation. If volumes are in specified range but error is displayed anyway, please call Technical Support.
-7032	Air in system during calibration of tubing.	Check buffer supply. Check for leakage of system (unintended air intake). Start a rinse program (WASH ONLY) and then retry calibration. Otherwise call Technical Support.
-7033	Date and time is outdated.	Set time and date to actual values.
-7034	Air intake during sample uptake although needle did not yet hit bottom of tube. Leakage of air into system in front of bubble sensor, liquid level has been overestimated or needle did not move to bottom (as fast as necessary).	Make sure foam on top of sample is not higher than 5 mm above liquid level. Check for leakage at transition of needle to tubing and tubing to bubble sensor. Touch CONT to process currently uptaken sample volume. Touch CANCEL to abort.
-7035	Air intake during sample uptake although needle did not yet hit bottom of tube. Leakage of air into system in front of bubble sensor, liquid level has been overestimated, or needle did not move to bottom (as fast as necessary). Uptaken sample has been processed. Portion of sample is remaining in tube.	Make sure foam on top of sample is not higher than 5 mm above liquid level. Check for leakage at transition of needle to tubing and tubing to bubble sensor.

Error code	Cause	Possible remedies
-7036	Plunger movement blocked because of column clogging, blocked tubing set, or any other cross-section constriction during output of the positive fraction. The positive fraction has not been eluted completely. Positive cells are still remaining in the system.	Touch CONT to discard the remaining positive cells into the waste bottle. To output positive fraction again at lower speed, exchange positive tube with an empty tube and touch RETRY.
-7037	Resuspended sample could not be taken up completely (no final air intake detected). Leakage of air into system behind bubble sensor. Portion of resuspended sample is remaining in tube.	Check for air inlet into system behind bubble sensor. Touch CONT to process currently uptaken sample volume. Touch CANCEL to abort.
-7038	Resuspended sample could not be taken up completely (no final air intake detected). Leakage of air into system behind bubble sensor. Portion of resuspended cells are remaining in tube.	Check for leakage of air into system behind bubble sensor. Readjust tubing connectors and verify correct fastening of columns.
-7039	Required volume cannot be provided in given Chill Rack.	Exchange rack with rack able to provide requested volume (e. g. Chill 50 instead of Chill 15). Touch RETRY to try again. Touch CANCEL to abort.
-7048	Miscalculation of diluter movement. Target position is negative.	Touch RETRY to use target position 0 instead of negative value for current diluter move. Touch CANCEL to abort. Please contact the Technical Support in all cases - also if the sample has been processed completely after touching RETRY.

## 11.1 Labeled schematics of the autoMACS Pro Separator

### Integrated computer for control of cell processing

All interactions with the computer are performed with a TFT color touchscreen (figure 11.1). A memory card is used to run all programs and to log processes.



Figure 11.1: Front view of autoMACS Pro Separator.

### Automated arm with ports for sample uptake and release of cell fractions

The automated arm (figure 11.1) is a computer-controlled part of the autoMACS Pro Separator. It holds two ports, one for sample uptake and release of the magnetically labeled, positive cell fraction and one for release of the non-labeled negative cell fraction. The automated arm moves in y and z directions. The ports are automatically washed in the autoMACS Pro Washing Station during and after the separation process to prevent cross-contamination between samples.

### Access covers

The front door (figure 11.1) is opened sideways to allow access to the parts of the fluidic system that require periodic maintenance by the user. This includes the autoMACS Columns, pump syringe, and upper valves. The bottom cover (figure 11.1) gives access to the lower valves and can be removed by pulling gently. The washing station cover (figure 11.1) is opened sideways giving access to the washing station, the peristaltic pump, and the tubing of the autoMACS Pro Washing Station.



### Fluid containers and fluid container baskets

Two baskets (figure 11.1) holding two fluid containers each, are located at each side of the instrument. Fluid containers are connected to the autoMACS Pro System with color-coded tubing and sensors at the bottle closures for fluid level control (figure 11.1).

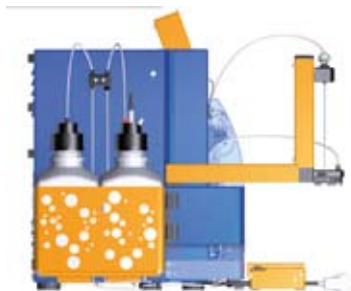


Figure 11.2: Left side view of the autoMACS Pro Separator.

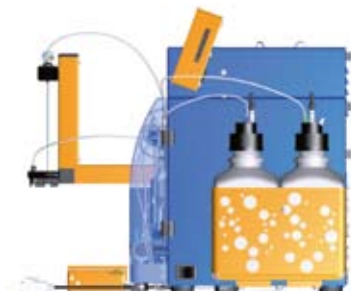


Figure 11.3: Right side view of the autoMACS Pro Separator.

### Plugs, connections and guidings

Sockets for the main plug (figure 11.4), the fluid sensor cables, the 2D code reader cable, and the MACS MiniSampler are installed at the rear of the instrument. Additional sockets are implemented for further instrument development. The main power switch is located at the right hand side of the instrument (figure 11.4). Several guidings at the rear and sides of the instrument ease the safe connection of tubings and sensor cables.



Figure 11.4: Rear view of the autoMACS Pro Separator.

### MACS MiniSampler, Chill Racks, and MACS Reagent Rack

The MACS MiniSampler (figure 11.5) can be loaded with one of three different cooling tube racks that carry cell samples and fraction collection tubes and the MACS Reagent Rack 4. The upper plate of the MiniSampler moves in an x-direction and aligns the tube openings with the port of the automated arm. The guiding of the MiniSampler is directly attached to the corresponding slot below the washing station. When attaching the MiniSampler sensor cable to the corresponding socket at the rear of the instrument the MiniSampler will be automatically detected. The type of tube rack carried by the MiniSampler is automatically recognized by the rack detector after starting the separation process. During operation, the tube rack should be covered with the MiniSampler lid that is connected to the lid guiding (figure 11.5). The MiniSampler can be disconnected from the autoMACS Pro Separator by pulling it up on the front side and pulling it towards the user.



Figure 11.5: Rear view of MACS MiniSampler with MACS Reagent Rack and Chill Rack 5.

### The cell separation unit

The central part of the cell processing unit consists of a magnet (figure 11.6) and two autoMACS Columns (figure 11.6). Once installed, the autoMACS Columns become part of a closed fluidic system and can be used for up to two weeks OR 100 separations, whichever comes first. Fluids are put through the fluidic system with the help of a syringe pump and five valves.

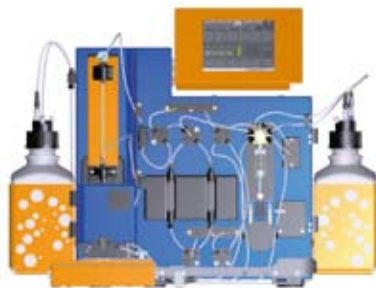


Figure 11.6: Front of autoMACS Pro Separator with access and bottom covers removed.

### **2D code reader (barcode reader)**

The autoMACS Pro Separator is equipped with a 2D code reader that uses lasers and powerful light-emitting diodes (LEDs) for illuminating the reading area. The 2D code reader is classified as a Class 1 laser product per standard IEC 60825-1:1993+A1:1997+A2:2001 (maximum output 116  $\mu$ W; wavelength 655 nm, pulse duration 1 ms). Please refer also to section 1.3.4 of the autoMACS Pro Separator user manual for associated warning and precautionary information. Refer to section 12.3 for information on installation of the 2D code reader.



Figure 11.7: Expanded view of the autoMACS Pro Separator 2D code reader ("barcode reader").

The technical specifications of the autoMACS Pro Separator and the peripheral devices are as follows:

# 11.2 Technical data and specifications of the autoMACS® Pro Separator

Parameter	Specification
Color	Blue / orange
Footprint	605 mm × 343 mm (w × d)
Footprint with MiniSampler	605 mm × 455 mm (w × d)
Height	392.5–454 mm (adjustable touchscreen)
Weight	25 kg
Input voltage (autoMACS Separator)	100–240 VAC, 50–60 Hz
Power consumption	200 VA
Fuses	2×T4A/250
Programs	12 preset
Sample volume (input)	0.25–50 mL
Sample volume (output)	0.5–52 mL
Column capacity	4×10 <sup>9</sup> cells / sample 2×10 <sup>8</sup> magnetically labeled cells / sample 15 mL of whole blood

Table 11.1: Technical data for the autoMACS Pro Separator.

Model	autoMACS Pro Separator
RS232-Interface (labeled "COM")	Pins 1: 4, 6, 9 NC Pin 2: RXD Pin 3: TXD Pin 5: GND Pin 7: RTS Pin 8: CTS
RS232-Interface (labeled "RS232/AUX") Not in use	Pins 1, 4, 6, 7, 8, 9: NC Pin 2: RXD Pin 3: TXD Pin 5: GND
RS232-Interface + DC-Output (labeled "RS232/BCR")	Pins 4, 6: NC Pin 1: Input Pin 2: RXD Pin 3: TXD Pin 5: GND Pins 7, 8: Shorted Pin 9: 5 VDC / 0.5 A
CAN-Bus + DC-Output (labeled "External CAN")	Pins 1, 4, 8: NC Pin 2: CAN-L Pins 3, 6: GND Pins 5, 9: 24 VDC / 2A Pin 7: CAN-H
AC-Output (labeled "Bottle Sensor")	Pins 1, 2, 3, 4, 5: 5 VAC / 10 kΩ Pins 6, 7, 8, 14, 15: GND Pins 9, 10, 11, 12, 13: Input
CAN-Bus (labeled "CAN1" or "CAN2")	Pins 1, 4, 5, 8, 9: NC Pin 2: CAN-L Pins 3, 6: GND Pin 7: CAN-H

Table 11.2: Pin assignment for autoMACS Pro Separator.

The autoMACS® Pro Separator is labeled as a protection class I instrument and must be plugged into a grounded power outlet, refer to section 1.

The main power supply cord and plug of the instrument shall comply with following specifications (USA and Canada only): UL listed and KAM cord, minimum type SJ, minimum 18 AWG, 3 conductors. Rated for a minimum temperature of 60 °C. Provided with grounding-type (NEMA 5-15P) attachment plug, rated 125 Vac, 10 A. Opposite end terminates in IEC 320 style connector, rated 125 Vac, 10 A. Conditions of operation: 15–30 °C with 0–85% humidity (non-condensing) at a maximum altitude of 2000 m. Supply voltage fluctuations up to +/-10% of the nominal voltage. Transient overvoltages present on the mains supply: category II. The instrument is suitable for rated pollution degree 2. Emission sound pressure level at workstation < 70 dB(A). The autoMACS Pro Separator is not specified for use in the cold

room. The autoMACS Pro Separator has been investigated by Underwriters Laboratories in accordance with the standards UL 61010-1, CAN/CSA- C22.2 No. 61010-1, and IEC 61010-2-081 and meets the intent of the directive 2004/108/EC (electromagnetic compatibility) and 2006/95/EC (low voltage equipment).

EMC:	EN 61326-1 EN 61000-3-2 EN 61000-3-3
Low voltage equipment:	EN 60825-1 EN 61010-1 EN 61010-2-081

Compliance was demonstrated by conformance to the following standards which have been listed in the Official Journal of the European Communities:

Compliance was demonstrated by conformance to the following FCC Rules of the Code of Federal Regulations:  
47 CFR §15, class B.

### 11.3 Technical data and specifications of the MACS® MiniSampler

Parameter	Specification
Model	MACS MiniSampler
Input voltage	24 VDC
Current	0.8 A
Sub D9 interface with shielding	Pin 1: NC Pin 2: CAN- Pin 3: GND Pin 4: NC Pin 5: +24 V Pin 6: GND Pin 7: CAN+ Pin 8: NC Pin 9: +24 V

Table 11.3: Technical data and pin assignment for MACS MiniSampler.

The MACS® MiniSampler is labeled as a protection class III instrument and must only be plugged into the connector labelled with “External CAN” of the autoMACS Pro Separator, refer to section 1.2.

Size without lid: 182 mm × 148 mm × 47 mm

Size with lid: 280 mm × 153 mm × 172 mm

Weight: 1.5 kg

The MACS MiniSampler is designed for operation with three different tube racks and a reagent rack.

Rack type	Slots	Maximum number of samples	Maximum sample volume	Maximum number of cells per tube
Chill 5	24 × 5 mL	6 (5 mL tubes)	2.5 mL	5 × 10 <sup>8</sup>
Chill 15	15 × 15 mL 5 × 5 mL	5 (15 mL tubes)	12.5 mL	2.5 × 10 <sup>8</sup>
Chill 50	6 × 50 mL 3 × 15 mL 3 × 5 mL	3 (50 mL tubes)	50 mL	4 × 10 <sup>8</sup>

Table 11.4: MACS Cooling Tube Racks: Chill Racks 5, 15, and 50.

Rack type	Slots
MACS Reagent Rack 4	4 × MACS Reagent vials

Table 11.5 MACS Reagent Rack 4

Conditions of operation: 15–30 °C with 0–85% humidity (non-condensing) at a maximum altitude of 2000 m.

The MACS MiniSampler is not specified for use in the cold room. The MACS MiniSampler has been investigated by Underwriters Laboratories in accordance with the standards UL 61010-1 and CAN/CSA -C22.2 No. 61010-1 and meets the intent of the directive 2004/108/EC (electromagnetic compatibility).



Compliance was demonstrated by conformance to the following standards which have been listed in the Official Journal of the European Communities:

EN 61326-1

EN 61000-3-2

EN 61000-3-3

Compliance was demonstrated by conformance to the following FCC rules of the Code of Federal Regulations:

47 CFR §15, class B.

## 12.1 Installation of new software

**Note:** Do not remove or insert a memory card while the instrument is turned ON. The memory card must remain in the unit. Removing the memory card during operation will abort all running processes.

After installing an autoMACS Pro software update it is necessary to calibrate the instrument as instructed below. The autoMACS Pro Separator contains various control boards. During initialization of the instrument with a software card, all of these components are automatically checked for the currently installed software.

### 12.1.1 Exchanging the software cards

- 1 Ensure the instrument is switched-off before proceeding.
- 2 Note the memory card slot at the right hand side of the touch screen.



Figure 12.1: Location of the memory card slot.

- 3 Remove the old memory card by pressing the black release button beneath the card slot.
- 4 Insert the new memory card.
- 5 Switch the instrument ON. A dialog box will appear prompting the operator to proceed if desired.
- 6 Select **Update** to confirm that you wish to proceed.



**Note:** During the panel software update process, the display may turn black or white or may freeze or flicker. This process may take several minutes. The progress will be indicated by an acoustic signal.

\* If warning "7030" is reported confirm by selecting **OK**.

\* If warning "7016" is reported select **Ignore**.



Figure 12.2: The user must confirm any update. The current software (panel software version) will be overwritten.

## 12.2 Calibration of the autoMACS® Pro Separator

The autoMACS Pro Separator is calibrated by using the two programs, **Calibr\_1** and **Calibr\_2**. Calibration 1 is recommended after installation of new software. Calibration 2 is always necessary when the pump syringe, the Diluter Valve, or the tubing are changed.

**Calibr\_1** is used to calibrate the settings of the needle arm (x, y, and z-axis), i.e., alignment of the needle arm with the washing station, the MACS Cooling Tube Racks, and the bottom detection sensor control.

**Calibr\_2** automatically calibrates the liquid volume control. This is crucial for the correct measurement and processing of the sample volumes. The fluidic system must be filled with buffer before commencing this step, i.e., the **Rinse** program must be performed.

### Calibration 1: Beginning the calibration sequence

- 1 Select menu **Option**.
- 2 Highlight **User settings** and select **Calibr\_1**.
- 3 Select **Run**.
- 4 Select **Calibrate** to proceed. The first of five (1/5) calibration steps will begin, namely, calibration of the washing station.

**Note:** If warning "-7016: Columns are not installed. Start column exchange program." appears, select **Ignore**.

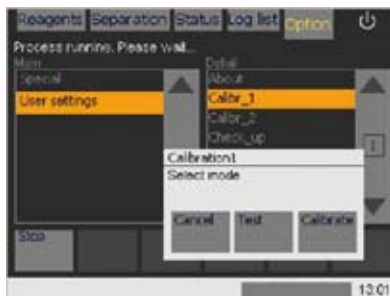


Figure 12.3: Performing calibration of the needle-arm: a 5-step calibration process.

### Calibration 1: Calibration of the washing station – step 1/5

- 5 Select **Calibrate** and then **Use** to proceed with calibration. The needle arm will automatically move towards the washing station and should be located directly above the center of the rear opening of the washing station.



Figure 12.4: Calibration of the washing station. To use already saved settings select "Use". To reset calibration settings (not recommended) select "Reset".

- 6 Select **Height** and check the central positioning of the uptake needle by using the buttons **Move up** and **Move down**. Select **Position** and adjust by using the **Move back** and **Move fwd** buttons. Select **Done** after making necessary adjustments.



Figure 12.5: Test calibration of the needle arm position in the washing station. The position of the uptake needle should appear as shown above.

7 Select **Save** to save the new configuration.



Figure 12.6: Saving new settings for calibration of the washing station.

### Calibration 1: Calibration of the tube rack – step 2/5

- 8 Select **Calibrate** to continue with the calibration process.
- 9 Place a MACS Cooling Tube Rack with sample tubes onto the MiniSampler. It is recommended to use a Chill 5 Tube Rack.
- 10 Select **Done** and then select **Use** (recommended) to start the calibration using current settings.
- 11 Check the central positioning of the uptake needle on the bottom of the tube by using the needle navigation buttons (**Move back, Move fwd, Height**). Ensure the needle is positioned at the bottom center of the tube in row A of the tube rack. Select **Done** to continue.



Figure 12.7: The tube must appear.

- 12 Select **Save** to store the new settings.

### Calibration 1: Calibration of the bottom detection – step 3/5

- 13 Select **Calibrate** to proceed with calibration.
- 14 Place a MACS Cooling Tube Rack onto the MACS MiniSampler.
- 15 Select **Done**.

- 16** Select **Use** to start the calibration at current position (recommended). Select **Reset** to reset the position to factory settings. The needle will position itself a fraction of a millimeter over the surface of the Chill Rack at a point equidistant to the tube coordinates A1, A2, B1, B2. It should not make contact with the plastic surface.



Figure 12.8: Bottom detection calibration. In the case of a Chill 5 Tube Rack the needle will be positioned midpoint (equidistant) to tube coordinates A1, A2, B1, B2.

**Note:** The uptake needle moves to the surface of the tube rack. The position of the needle tip is correct if a sheet of paper can be easily slid between the needle tip and the surface of the tube rack.

- 17** To adjust the position, select **Change** or select **Done** if the current settings are correct.
- 18** If **Change** was selected (see above), use the **Up** and **Down** navigation buttons to adjust the position as required.

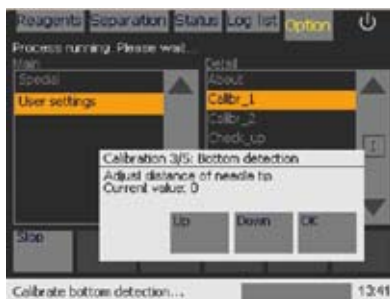


Figure 12.9: If necessary, adjust the z-axis of the needle tip using “Up” and “Down” navigation tools.

- 19** Select **OK** and **Done** to save the current configuration.

### Calibration 1: Calibration of the x-axis – step 4/5

- 20** Select **Calibrate** to proceed with calibration.
- 21** Place a MACS Cooling Tube Rack and a MACS Reagent Rack 4 onto the MACS MiniSampler. It is recommended to use a Chill 5 Tube Rack.
- 22** Select **Done**.

**Note:** It is possible that the stored calibration value is zero!

In this event, step 23 will be skipped. Continue with step 24.

**23** Select **Use** to start the calibration at current position (recommended). Select **Reset** to use factory settings.

**24** If necessary, adjust the central positioning of the uptake needle by using the needle navigation buttons (**Move left**, **Move right**, **Height**). The uptake needle must be positioned above the center of the tube in row A of the tube rack.



Figure 12.10: The uptake needle should be positioned as shown above.

**Note:** Due to mechanical limits of the MiniSampler the correction potential is limited. Exceeding these limits is reported by the message "standard range exceeded".

Calibration outside the standard range is not recommended. The outer positions (reagent vial and sample row 6) will not be reached properly. Labeling in sample position 6 will be prohibited. In this event contact Technical Support (refer to section 13).



Figure 12.11: Adjust MiniSampler position.

**25** Select **Save** to store new settings.

### Calibration 1: Test current calibration settings – step 5/5

**26** Select **Test** to test new configurations. The autoMACS Pro Separator will perform a complete test of Calibration 1 settings.



Figure 12.12: It is important to test the new calibration settings. To navigate back through the calibration steps, select "Previous".

- 27 Place a MACS Cooling Tube Rack onto the MACS MiniSampler.
- 28 Select **Use** to start the calibration at the current position (recommended). Select **Reset** to reset the position to factory settings.
- 29 Place a MACS Reagent Rack 4 onto the MACS MiniSampler.
- 30 Select **Done**.
- 31 Check all approached positions by using the needle navigation buttons **Move up** and **Move down**, then select **Done**.
- 32 If any errors or misalignments were noted, repeat the entire process.

### Calibration 2

- 1 Select menu **Option**.
- 2 Highlight **User settings** and select **Calibr\_2**.
- 3 Select **Run**.



Figure 12.13: Performing "Calibration 2" by selecting program "Calibr\_2".

- 4 Select **Calibrate**. The calibration is performed automatically.
- 5 Press **Save** to finish **Calibr\_2**.

## 12.3 Installation of the 2D code reader (barcode reader)



Figure 12.14: Contents of the autoMACS Pro Separator upgrade kit.

**Note:** Carefully read the chapter 1 before installing the 2D code reader.

If the autoMACS Pro Separator is not provided with the optional 2D code reader, an autolabeling package is required for installation. Please contact Miltenyi Biotec or your local distributor for further information. The autolabeling package contains:

1× 2D code reader  
1× MACS Reagent Rack 4  
1× Autolabeling Software Tools for installation (a 2 mm allen key, a headless screw, an optical frame for the 2D code reader window in the washing station cover, and a screw driver for connecting the reader to the outlet at the rear of the instrument).

- 1 Switch OFF and unplug instrument.
- 2 Open and remove the washing station cover.



Figure 12.15: Removing the washing station cover

- 3 Remove the peristaltic pump tube at the right hand side of the washing station.



Figure 12.16: Remove the peristaltic pump tube

- 4 Unscrew the thumb screw. Pull to remove washing station from instrument. Take care to clean spilled fluids with ethanol or disinfectant. Remove the washing station.



Figure 12.17: Unscrew the thumb screw.

- 5 Note the position of the 2D code reader port.



Figure 12.18: The position of the 2D code reader port.

- 6 Guide the cable of the 2D code reader underneath the lower front access cover at the left-hand side. Make sure that 2D code reader cable is located in-between the peristaltic pump tubes.



Figure 12.19: Make sure that 2D code reader cable is located in between the peristaltic pump tubes.

- 7 Insert 2D code reader pin into corresponding opening.





Figure 12.20: Inserting the 2D code reader.

- 8 Fix the connection by fastening the headless screw in the tapped bore hand-tight with help of a 2 mm allen key.



Figure 12.21: Fastening with the allen key.

**Note:** Please check if headless screw is already present in the tapped bore. Otherwise use headless screw provided with autolabeling package.

- 9 Reinstall the washing station. Make sure that the cable is installed straight and is guided between the peristaltic pump tubing.



Figure 12.22: Reinstall washing station.

- 10 Remove the black plastic square from the 2D code reader window of the Washing Station Cover (not shown). Snap in black optical frame. Reinstall and close the washing station cover.
- 11 Guide the 2D code reader cable underneath the instrument. Connect the 2D code reader plug with the corresponding outlet at the back of the instrument labeled "RS232/BCR". Use the screw driver to fasten the screws of the connector.

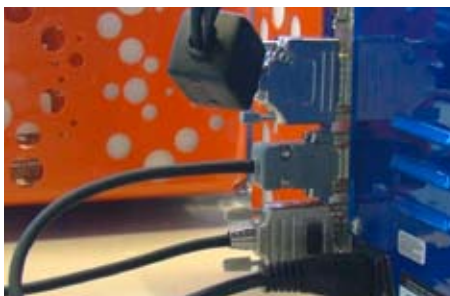


Figure 12.23: Location of the cable sockets at the back of the autoMACS Pro Separator

- 12 Plug in and switch on instrument.
- 13 Select menu **Option**. Highlight **User Settings** and **O\_bcr**. Press **Run**.

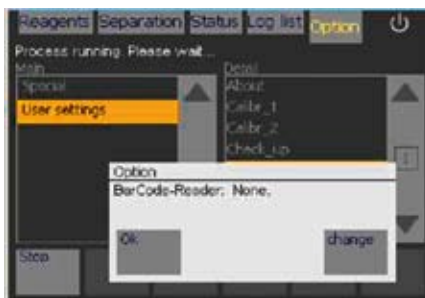


Figure 12.24: Setting up the software to recognize the 2D code reader.

- 14** Select **Change**.
- 15** Select **Keyence**.
- 16** Switch OFF instrument. Wait 5 seconds and switch ON instrument again.
- 17** Replace the cover.

Miltenyi Biotec offers a full range of customer technical support options for your autoMACS Pro Separator.

For support and technical questions, or if you think your autoMACS Pro Separator is defective, please contact your local Miltenyi Biotec representative or Miltenyi Biotec's technical support team:

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**Air filter:** Hydrophobic 0.2  $\mu\text{m}$  air filter attached to the bottle closure. Used to vent the bottle and—at the same time—prevent contaminants from entering the fluid bottle.

**Air filter connector:** Luer-to-thread connector for attaching the air filter to the threaded bottle closure vent.

**APC:** Allophycocyanin

**autoMACS Columns:** Specifically designed autoMACS Columns; reusable for two weeks or for 100 separations within these two weeks.

**autoMACS Column 1:** First autoMACS Column in which labeled cells are retained during positive selection and depletion programs. The autoMACS Column 1 occupies the left slot of the black magnet cover.

**autoMACS Column 2:** The second autoMACS Column is used during double selection programs. The autoMACS Column 2 occupies the right slot of the black magnet cover.

**autoMACS Pro Separator:** Automated magnetic cell separator, also referred to as device or instrument.

**autoMACS Running Buffer:** Sterile and ready-to-use solution for cell separation and washing programs. The tubing connector is color-coded blue.

**autoMACS Pro Washing Solution:** Sterile and ready-to-use solution for washing and special rinsing programs. The tubing connector is color-coded green.

**Bottle closure:** Vented screw-on closure with fluid uptake ports / canules and distribution tubes. The bottle closures contain fluid sensors and are equipped with sensor cable connectors.

**Column Connector:** Luer-to-thread connector connecting the autoMACS Columns to the fluidic system.

**Column substitute:** Column without spheres that replace the autoMACS Columns for long-term storage and shipment. Column substitutes cannot be used for cell separations.



**Depletion:** Isolation of target cells by labeling all cells other than the target cells (non-target cells?) with MACS MicroBeads and subsequently performing a MACS Separation. The non-labeled fraction contains the target cells.

**Deplete:** Depletion program, standard mode: a normal cell deposition rate is used. The non-labeled cells are eluted in row B of the sample rack. This is the optimal program to be used in combination with MACS Cell Isolation Kits or to achieve the highest recovery rate of untouched cells.

**Depletes:** Depletion program, sensitive mode: a slow cell deposition rate is used (1 mL/min). The non-labeled cells are eluted in row B of the sample rack. This program is optimized for depletion of those cells that weakly express the antigens used for magnetic labeling, or to achieve optimal purity of the untouched cell fraction.

**Depl05:** Special depletion program: the cell deposition rate is 0.5 mL/min. The non-labeled cells are eluted in row B of the sample rack. This program is used for strong depletion of those cells that weakly express the antigens used for magnetic labeling, or to achieve the highest purity of the untouched cell fraction. However, choosing Depl05 might result in a reduced recovery of the target cell fraction.

**Depl025:** Special depletion program: the cell deposition rate is 0.25 mL/min. The non-labeled cells are eluted in row B of the sample rack. This program is used for very strong depletion of those cells that weakly express the antigens used for magnetic labeling, or to achieve the highest purity of the untouched cell fraction. However, choosing Depl025 might result in a reduced recovery of the target cell fraction.

**FITC:** Fluorescein isothiocyanate

**Fluid container:** 1.5 L bottles holding the fluids for operational use of the autoMACS Pro Separator. Fluid sensors monitor the fluid levels in the containers for Running Buffer, Washing Solution, and waste via electrolyte conductivity. The fluid level in the container for the 70% ethanol cannot be monitored as no electrolytes are present in the solvent.

**Fluid sensors:** This sensor type measures electrolyte conductivity and is an integral part of the bottle closures of the fluid containers for waste, Running Buffer, and Washing Solution.

**Fluid sensor cable:** Cable connecting the fluid sensor to the autoMACS Pro Separator. The sensor cable connectors on the bottle closures are color-coded: red for waste, green for Washing Solution, and blue for Running Buffer.

**Fraction collection tube:** 5 mL, 15 mL, or 50 mL plastic sample tubes to collect the positive and negative fractions. The process has been optimized using tubes from BD Falcon.

**Front door:** The front door opens sideways, giving access to the autoMACS Columns, pumps, valves, washing station, and tubings.

**MACS Technology:** Technology developed by Miltenyi Biotec for immunomagnetic labeling and subsequent separation of cells or biomolecules in a high-gradient magnetic field.

**MACS MicroBeads:** Super-paramagnetic particles conjugated to antibodies for magnetic labeling of cells or biomolecules.

**Magnet cover:** Black cover surrounding the magnets. The magnet cover is located in the center of the fluidic system and has slots for the autoMACS Columns.

**Memory card:** Removable compact flash/SD RAM card containing the autoMACS Pro Programs.

**Memory card slot:** Slot located on the right hand side of the autoMACS Pro Touchscreen, giving access to the memory card. The memory card should be removed by trained personnel only.

**Negative fraction:** Sample fraction containing the non-labeled cells that pass through the autoMACS Column while the column is placed in the magnetic field.

**PE:** Phycoerythrin

**PerP:** Peridinin chlorophyll protein

**Ports:** The automated arm carries two ports: the port located proximal to the instrument is designed for computer-controlled fluid detection and distribution such as magnetic labeling, sample mixing, sample uptake, and release of the magnetically labeled fraction while the port in the front releases the non-labeled fraction.

**Positive fraction:** Sample fraction containing the cells labeled with MACS MicroBeads. These cells are retained on the column while the column is placed in the magnetic field. The cells are eluted from the column after the column has been removed from the magnet.

**Positive selection:** Process of isolating cells by labeling the target cells with MACS MicroBeads and performing a MACS Separation. The labeled target cells are eluted in row C of the tube rack.

**Possel:** Positive selection program in standard mode using one autoMACS Column. The target cells are eluted in row C of the sample rack. This program is used for cells with normal to high frequency and with normal antigen expression.

**Possel\_s:** Positive selection program, sensitive mode, using one autoMACS Column. The target cells are eluted in row C of the sample rack. This program is used for cells with normal to high frequency which weakly express the antigens used for magnetic labeling, or to achieve the highest recovery of the target cells.

**Posseld:** Positive selection program, normal mode, using both autoMACS Columns. The target cells are eluted in row C of the sample rack in a volume of 0.5 mL. This program is used to isolate rare cells or to achieve a higher purity of the target cells.

**Posseld2:** Special positive selection program, normal mode, using both autoMACS Columns. The target cells are eluted in row C of the sample rack in a volume of 2 mL. This program is used to isolate rare cells from whole blood, cord blood, or large cell samples, and to achieve a higher recovery of the target cells.

**Posselds:** Positive selection program, sensitive mode, using both autoMACS Columns. The target cells are eluted in row C of the sample rack in a volume of 2 mL. This program is used to isolate rare cells that weakly express antigens used for magnetic labeling (e.g. CD133).

**Posselwb:** Special positive selection program using both autoMACS Columns for the isolation of cell subsets from whole blood. The target cells are eluted in row C of the sample rack.

**Running Buffer bottle:** Container for Running Buffer. The bottle closure is equipped with a fluid sensor. The closure, the fluid sensor cable, and the tubing connector are color-coded blue.

**Safe solution:** Solution of 1% (w/v) sodium hypochlorite in distilled water used to decontaminate the autoMACS Pro fluidic system with the Safe program. The safe solution is fed into the system from a 50 mL tube. Upon completion of the Safe program, the fluidic system contains Running Buffer.

**Storage solution:** Solvent used during the Sleep, Store, Safe, and Column Exchange programs to minimize the contamination risk. The fluidic system of the autoMACS Pro Instrument is filled with MACS Storage Solution (70% ethanol).

**Storage solution bottle:** Container for 70% ethanol. The bottle closure, the fluid sensor cable, and the tubing connector are color-coded black.

**Store program:** Prior to long term storage, the Store program should be applied. During this procedure the autoMACS Columns are replaced with substitutes. Upon completion of the Store program, the fluidic system contains 70% ethanol.

**Syringe pump:** Computer-controlled high precision syringe pump with Teflon® seal plunger that drives fluids through the autoMACS Pro fluidic system.

**Touchscreen:** High resolution TFT color touchscreen located on top of the autoMACS Pro Separator. The touchscreen is used to operate and monitor the instrument through on-screen menus.

**Tubing connector:** Plastic threaded connector with square nut used to connect the tubings to the bottle closures, the columns, the pump, or valves.

**Tubing system:** Permanent set of Teflon® tubing through which fluids circulate in the autoMACS Pro Separator fluidic system.

**Tube racks:** Three different acrylic tube racks are provided with the instrument. They are designed for optimal positioning of sample tubes and fraction collection tubes at the ports of the automated arm. They contain a coolant allowing to pre-cool the racks in the refrigerator for cooling of the cells during the separation process. The racks have four tube positions for each sample. Position A holds the sample tube containing the starting material. Position B holds the tube for the non-labeled fraction. Position C holds the tube for the magnetically labeled fraction. Position D is auxiliary. For details, refer to the table in section 4.1.2.

**Washing Solution bottle:** Container for autoMACS Pro Washing Solution. The bottle closure is equipped with a fluid sensor. The closure, the fluid sensor cable, and the tubing connector are color-coded green.

**Waste container:** Container for waste fluid. The closure is equipped with a fluid sensor. The closure, the fluid sensor cable, and the tubing connector are color-coded red.

**Whole Blood MicroBeads:** MACS MicroBeads developed for isolating target cells directly from whole blood by using the autoMACS Pro Separator.

**Wrench:** Black wrench used to tighten and loosen tubing connections.